STUDIES ON BIOCHEMICAL INVESTIGATION ON SOFT ROT OF ZINGIBER *OFFICINALES* ROSE (GINGER)

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DECLARATION

I here by declare that with guidance and suggestions received from my Supervisor, Prof. M.C. Kanchan, Botany Department, Bundelkhand University, Jhansi, this Thesis is my own unaided work. It is based upon the research work carried out at Bipin Bihari Maha Vidhyalaya,Bundelkhand University, Jhansi,U.P, Indian Agricultural Research Institute, New Delhi. Dept. of Botany. Jabalpur Krishi Vishwa Vidhyalaya, Jabalpur M.P., Dept. of Botany.

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1ST SEPTEMBER 1992
JHANSI, U.P.

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Supervisors Certificate.

I hereby certify that this thesis entitled, "Studies on biochemical investigations on soft-rot of Zingiber officinale Rose, submitted to the Bundelkhand University Jhansi for the degree of Doctor of Philosophy, is an original piece of research work, carried out by Mrs Archana Gupta under my guidance and supervision, for more than 2. academic years & 200 days lab work.

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SECTION A

CHAPTER - I

THE HOST - ZINGIBER *OFFICNALES* (ROSE)

Zingiber officinales Rose (Ginger) Trans. Linn. SOC.VIII (1807) 348-Plate - 944 is named variously as in Arabic : Zanjabil; Brazil : Mangaratia; Zingiber, Burma : Khyenseing; Canarese : Alla, Ardraka, Hasisunthi, Sunti, Vanasunthi; Cantonese: Kon Kenng Kenug p'i; Catalan : Gengibra; Chinese: Chiang p'i, Kan Chiang, Kiang, Sheng Chiang; Danish : Ingefaer; Deccan : Ala; Dutch: Gember; Ewe : Engkrama, Engkrawnsa; Fanti : Akakadur, Tsintsinmin; French : Gingembre, Herbean gingembre; Fulah : Niamakubedi; Ga : Engelfail, Kakaotehofang; German : Ingwer; Mansa : Chitta Afu; Hungarian : Gyoember; Ilocans : Baseng; Italian : Zenzero, Zenzevero; Konkani : Alenm; Krepi : Engkrama; Frobo : Odzahwi; Kyerepon : Abrofoyisa ; NKrabodo; Malay : Alea, Baring, Haliabera; Malaya : Keong phee, Kon Kenng; Malinke : Niemekon; North West Provinces : Ada; Norway : Ingefarer; Persian : Shangabir, Zanjebil; Protugese : Gengibre, Gengivre, Gingibre; Romanian : Ghimber, Imbir; Russian : Imbir;Sinhalese: Inguru; Sonsson : Sarahu diabata; Tagalog: Luya; Tulu : Sunthi; Twi : Akekaduru, Kakaduru, Kekeduru; Uriya : Ardroko; da, Sunthi; Zambales : Layal, Pangas; Assam : Ada; Bengal : Ada, Bombay :Adu, Ale;Punjab :Ada, Adrak;Tamil :Allam, Arttiragam, Attiradam, Injii, Kulumamulam, Kodataram, Mamppu, Sangai, Sigaram, Singaveram, Singiveram, Sukku, Sundi, Ubugallam, Verkkombu;English :Ginger;Hindi; Ada, Adrak; Sanskrit : Anupama, Apakrishnaka, Ardraka, Ardrashaka, Chandrakhya, Gulmamula, Kandara, Katubhadra, Katukkata, Machhaka, Mahija, Mulaja, Rahuchhana, Saikateshtha, Sharuga, Shringahara, Sushakaka, Vara;Telugu : Allamn, Ardrakarme, Mahanshadaum, Sonti, Sunthi, Srigaberamu.

Plant is herbaceous, rhizomatous, perennial, reaching upto 90 cm in height under cultivation,. Rhizomes are aromatic thick lobbed, pale yellowish differing in shape and size according to their cultivated varieties,. The herb develops several lateral shoots in clumps which begin to dry when the plant matures.

Leaves are narrow, distichous, sub-sessile, linear-lanceolate when fully developed measuring 17.0 cm x 1.8 cm in size dark green, gradually tapering to form a slender tip, flowers in spikes greenish yellow with a small dark purple or purpish black tip. Stamens dark purple, rather stouter than the corolla.

Ginger has been under cultivation in India from times immemorial.Cultivation of ginger in India has been worked out by Nair (1969)Panlose (1972,1973) Muralidharan (1973) and

it has also been established that ginger is of great economic value and a cash crop Panlose (1971).The plant does not occur in wild state. Several commercial types are recognised in cultivation. They are generally named after the localities where they are grown as mentioned earlier.

Ginger is grown both as a pure crop and as an intercrop. It may be grown in rotation with rice, gingelly, Samai (Panicum miliare) etc. If irrigation facilities are available, ginger may be rotated with vegetables, groundnut, ragi and maize.

Ginger requires a warm and humid climate. The plant thrives well from the sea level upto an altitude of 1,500 m in the Himalayas, the optimum elevation being between 300 m and 900 m. A well distributed rainfall (150 - 300 cm) during the growing season and dry spells during land preparation as well as before harvesting are required for large scale cultivation of the crop. Cold climate during the resting period does not affect the crop. It can be grown in a wide range of well drained soils of at least 30 cm. depth; heavy laterite loam soil of Anantpur District (Andhra Pradesh), red laterite of Kottayam and Malabar regions and clayey loam of Wynaud District in Kerela are found suitable for the crop; it also grows well in rich black and clayey soils under irrigation in Baruasagar at Jhansi district, Karnataka, Maharashtra and Andhra Pradesh. According to Mudaliar; Gupta(1926, 73) ; Kannan and Nair (1965) in Wealth of India, the crop prefers light shade for good growth but shade is not absolutely necessary.

In Baruasagar the crop is grown as an irrigated crop and planting is done in March. The crop is harvested in stages depending upon the prevailing demand. The crop becomes ready for harvesting from the fifth month after sowing. Shriveling, yellowing and withering of the leaves accompanied by drying and lodging of the aerial stems, indicate maturity. The crop is harvested about seven months after planting in December - January in South India raised from April - May sowing, and in September-November in Baruasagar and East India raised from March sowing. The rhizomes for seeds are allowed to remain unearthed and are lifted 60 days later. The crop for the production of dry ginger may be harvested later but not beyond 260 days after planting according to Panlose (1972) as further delay not only increases the fiber but also decreases the percentage of volatile oil.

For cultivation raised beds 3 m x 1 m, are laid out at a distance of 30-45 cm from each other, small shallow pits for planting are then made on the beds at a spacing of 15 or 20 cm x 22 cm. The beds are smaller in sloppy areas. A handful of cattle manure is applied to each of these pits. The optimum spacing for planting of ginger is 25 - 45 cm, between the rows and 15-20 cm between the plants in a row. The seed rhizome should be 20-30 gm in weight

with atleast two sprouted eye buds. It is placed 3.5-5.0 cm deep in the pits and the soil is pressed over it, this is followed by light irrigation, Mulching these beds twice with green manure is an important part of cultivation.

Ginger is an exhausting crop and requires heavy manuring. Well rotten cattle manure or compost at the rate of 25-30 tones per hectare is applied at the time of planting . Mulching the bed is very important,. First mulch conserves water and helps rhizomes in germination.

The quantity of inorganic fertilizers depends upon the fertility of the soil and the amount of organic manure applied to the crop. Ready made fertilizers for the crop are available in the market under the name "Ginger Mixture", consisting of N, P & K in the ratio of 1:1:2 respectively. About 750 kg of the mixture is applied per hectare, one and a half as a basal dressing and the other half 2 months after planting.

The composition of ginger varies according to the type and the agro climatic conditions under which it is grown. According to the analysis made by Sen Gupta & Rai (1971) in Nutritive Value of India foods, a bazar sample of green ginger gave the following values: moisture- 80.9, protein-2.3, fat - 0.9, fiber - 2.4, carbohydrates - 12.3, and minerals -1.2%, Ca-20, P-60, and Fe 2.6 mg/100 gm. Ginger contain s traces of Iodine and Fluorine (I-0.82 ppm and F-2.0 ppm in one sample).

The vitamins present in green ginger are : Thiamine-0.06, Riboflavin - 0.03, Niacin - 0.60; and vitamin C - 6.0 mg/100 gm. The value reported for caroteine in the fresh rhizome is 40 mg/100g.

The studies of Connell & Mclachian 1972, Rao 1956 & Parry concluded that Ginger contains small quantities of glucose, fructose and sucrose; raffinose is probably present in traces. The principal carbohydrate of the rhizome is starch. Besides starch, rhizome are reported to contain pentosan. According to the studies of Srivastava, 1962-63 & Rao 1966 Ginger contains 1.60 - 2.44 % nitrogen on dry basis, of which non protein nitrogen accounts for roughly one third. Extraction of the freeze dried and powdered rhizome by the conventional protein solvent showed that Albumin, Globulin, Prolamine and Glutelin formed respectively 35.6, 16.9, 11.0 and 17.9% of the total protein, 18.6 % of the total protein remain unextracted. The free Amino acids present in Ginger include Glutamic Acid, Asparitic Acid, Serine, Glycine, Thromine, Alamine, Glutamine, Arginine, Y-amino Butyric Acid, Valine, Phenyl Alanine, Asparagin, Lysine, Cystine, Histidine, Leucine, Protine and Pipecolin Acid.

The characteristic pleasant and aromatic odour of ginger is due to an essential oil.The oil is generally obtained from unscraped ginger. The essential oil derived from the dried ginger, known in trade as "Oil of Ginger" is greenish to yellow in colour, mobile (viscous on ageing) with the characteristic warm and aromatic odour, but not the pungent flavour of the spice.

The rhizome is valued in medicine as appetiser, laxative stomachache, aphrodiasic, cuminative, useful in the diseases of the heart and the throat, dyspepsia inflammations, "Kapha" and "Vata", bronchitis, asthma, vomiting, pains, should not be used in leucoderma, anaemia, strangury, leprosy, ulcers, fevers, burning sensations and diseases of the blood. Ginger rhizome is alexiteric, improves taste, useful in indigestion, elephantiasis, piles, eructations and abdominal troubles.

Ginger is a well known popular remedy for snake bite and scorpion sting, but it is not antidote to either snake venom (Mhaska and Caius) or scorpion venom (Caius and Mhaska).

The essential oil from rhizome was studied by Sanjivarao, Sudborough and Watson (1925) (Jorn. Ind.Inst. SC VIII (A) 1925) and later by Mondgill (1928) (Jorn. Ind. Chem. SC V, 1928).

The synonyms, uses and references has been quoted from the journal of the "Medicinal plants of India Vol. IV".

A number of diseases of Ginger has been listed by Sharma & Jain (1977a).

CHAPTER - II

THE PATHOGEN - PYTHIUM aphanidermatum (EDSON)FITZPATRICK

Pythium aphanidermatum (Edson) Fitzpatrick, causing soft rot of ginger is common in most of the ginger growing tracts of India. Buttler (1907) first noticed this serious disease of ginger in Surat (Gujrat) caused by a species of Pythium, which was identified as Pythium gracile Schenk.

Buttler (1918)considered that though the species in question had been provisionally identified as P. gracile, the species isolated from ginger differs from the typical P. gracile described by Schenk. He further considered that this species might be a new one. Subramaniam (1919) described the soft rot of ginger from Pusa and the pathogen considered by him to be the same as reported by Buttler (1907, 1918) but it was identified as P. buttler Subram, a new species. Later Mitra & Subramaniam (1928) from their results and inoculation experiments, came to the conclusion that P.buttler is a strain ofP.aphanidermatum.Buttler & Bisby (1931) considered P. buttleri & P. gracile to be identical with P. aphanidermatum.

Considering the mycological history and taxonomy Prinsheim in 1858 placed the genus Pythium in Saprologuiaceae. Later the relationship with other Oomycetes was established and the genus was included in a new family Pythiaceae by Schroter in 1897.

Tammen in (1961)working in Argentina produced a good taxomic treatment for 17 sps. His work is well illustrated and in general follows Middleton (1943) concept of the genus. Waterhouse complied the original description and illustration of all described species and published a key to the genus in 1967.

Bateman (1959) Grover and Dutta (1973) described Pythium aphanidermatum's morphology and physiology It's hyphae 2.8-7.3 (4-6) in dia, hyalin and non septate except in fruitification, sporangia inflated filamentous, branched or unbranched to varying length, usually forming complexes Zoospores , laterally bicilliate, 12 x 7.5 µ spherical terminal, rarely intercalary 22µ india. Antheridia usually monoclinous but also diclinous typically intercalary though often terminal, one or 2 per ooganium, barrel shaped or dome shaped, suborbicular, becoming cylindrical or broadly clavate, 9-11 x 10-14 µ,conspicuous penetration tube. Oospores apterolic, single moderately thick wall, 17-19 µ in dia; germination by tube. The culture isolated by the author from infected rhizome had the same values.

CHAPTER - III

SOFT ROT

Soft rot of ginger caused by Pythium aphanidermatum (Edson) Fitz is of common occurrence in the ginger growing tracts of India. Buttler (1907) as already reported in previous chapter first noted in Surat (Gujrat); Subramaniam (1919) described it from Pusa (Bihar),.

The disease is epiphylotic and causes enormous loss to the growers. Buttler (1907) estimated the loss in a single village in Surat to be more than Rs. 10,000 . Mc Rae (1908,1909) estimated the loss upto 80% in exceptionally wet years in Bengal. The author has also found that about 80% of the crop is lost due to this disease during the study period. Infected plants either die early producing no rhizome or there may later recover partially. The symptoms of the disease were first evident on leaves which show slight paleness as compared with the normal healthy ones. Then yellowing was noticed on leaves, particularly at the tips which gradually spread down the leaf blade & leafsheath more along the margins than along the centre. As a result in the initial stages, while the margins, had already became yellow, the center remained green. The yellowing was followed by withering and drying and the dead areas gradually extended towards the leaf sheath following the path of yellow discolouration. The dead leaves gradually drooped and hang down along the stem till entire shoot becomes dry. At the same time basal portion of the plant between the point of attachment with the rhizome and soil surface showed a pale translucent brown colour and later became water soaked, due to destruction of parencymatons tissue to such an extent that the whole shoot could be easily pulled out but the stem usually does not fall off. Soft rot extends from the collar region to the rhizomes, which first become discoloured and gradually decomposed forming a watery mass of putrefying tissues enclosed by the tough rind of rhizome. The fibro vascular strands are not affected and they lie isolated among the decaying mass. The roots arising from these affected regions of rhizomes also showed typical rotting and softening. The infected plant fail to produce any new rhizome. Soft rot of ginger has been reported from Rajasthan by Kothari (1966) Lin et al (1971) have reported soft rot of ginger from Taiwan Trujillo (1963) have also described the rhizomes rot of common ginger.

Disease generally occurs either during sowing where the shoots arising from such rhizomes undergo damping off during seedling stage or if infection develops during the later stage death occurs in adult plants and such plants fail to produce rhizomes, however the author has found that generally at Baruasagar early damping off is more common.

Beside damages in the field, rotting is also noticed in the rhizomes collected from the infected crop and kept in storage, particularly were preserved in pits (Thomas (1938), 1939). Mycelium of the casual organism, is found in the underground parts of affected plants, occasionally in the stem and the leaf - sheath. The disease is wide spread in the field.

The hyphae of P. aphanidermatum penetrate the cells freely, forming usually irregular swellings and the rotting is usually in advance of penetration. The non septate mycelium of the branching hyphae forms long tapering zoosporangia by the apical swelling of hyphae, when the branches extend to the outside where there is plenty of moisture or when they are placed in water. After certain internal changes, each apical swellings blows out into a vesicle in which the protoplasm migrates. In the vesicle the protoplasmic content undergoes division to form small segments, each of which is transformed into a zoospore, which escapes from the side from the rupture of the cell wall. Zoospores after swimming for some time rest to form walled structure which germinate directly to produce mycelium. Sexual reproduction takes place within the tissue of the infected plants. Oogonia are formed generally on short lateral stalks but may also be inter calary antheridium is paragynous Oospores are produced singly in the Oogonia.

Sporangia are developed terminally also widely intercalary. zoospore formation is rare. Sporangia germinate directly by producing germ tubes.There are 2 ways in which the disease is carried over or propagated first through diseased rhizomes and secondly through Oospores in the soil. Mc Rae (1911) considered the use of the diseased rhizomes to be the principle factor in the dissemination of the disease. The fungi are common soil dwellers and they can probably live as saprophytes for a long time. The infected rhizomes contain mycelia and fruiting bodies inside. Oospores have also been found in the scales or preserved rhizomes (Thomas 1938).

CHAPTER IV

REVIEW OF THE LITERATURE

Ginger (Zingiber officinales Rose) is an economically important crop of Bundelkhand region particularly Baruasagar is the well known centres of ginger cultivation. In these areas soft rot of ginger is a serious problem.

The disease, soft rot of ginger was first reported by Buttler (1907) from Surat (Gujrat). Subramaniam (1919) described it from Pusa (Bihar). Ginger is commonly damaged by soft rot caused by species of Pythium. The important species which cause soft rot are P. aphanidermatum (Eds). Fitz reported by Buttler & Bisby (1931) from Gujrat, Shahare & Asthana reported it from M.P. (1962), P. myriotylum Drechser was reported to be the casual organism by Middleton (1943) from Gujrat, while Patel et al (1949) & Bhagwati (1960) from Bombay & Pune reported the same as the casual organism. P. aphanidermatum has also been reported to be responsible for the soft rot disease in Ceylon; particularly in plant raised from seed rhizomes received from Surat (Park, 1941). Sharma (1977), Sharma & Joshi(1977)also reported it for storage and freshly harvested rhizome from Jabalpur M.P.Pythium vexans de Barry was also found to cause the same disease in Wynad (Tamil Nadu) at an elevation of 1000 m. and above by Ramakrishnana (1949). The other species reported to have caused disease of ginger are P. buttleri Subram by Subramaniam (1919) & Thomas (1938) & P. monospermium pringsh by Buttler (1907)& Middleton (1943) .

The genus Pythium includes a number of readily recognized species with wide distribution and host ranges. The taxonomic position of the fungus and its relationship to other Phycomycetes were well established during the latter part of the 19th Century. In the early 1900s pathologist found Pythium species consequently associated with root diseases and it soon became apparent that these fungi were important plant pathogen. Certainly, not all isolates of Pythium species are capable of causing disease of plants, many are soil borne pathogens, that cause serious economic loss to a wide variety of hosts while others are more limited in host and geographic range or affected plants only under moisture. Several species of Pythium have been reported. They are P. aphanidermatum (Edson) Fitz (Mitra & Subramaniam, 1920) P. buttleri Subram. (Thomas, 1938), P. complecteus bronu (Park, 1934), P. deliense Mems Haware & Joshi,(1974a) P. grascile de Barry Schenk (Buttler, 1970), P. gramnicolum Subram, (Park, 1935), P. myriotylum Drescher (Uppal 1940; Park, 1941; Buttler, (1942), P. vexans de Barry (Ramakrishnana, 1942), Buttler & Bisby (1931) considered P. Buttleri & P. gracile to be identical with P. aphanidermatum. In Madhya

Pradesh, P. aphanidermatum (Haware & Joshi,(1972)), P. deliense (Haware & Joshi, 1974) & P. myriotylum (unpublished IMI 20669) are reported to be responsible for soft rot.

K.C. Shahare & R.P. Asthana in 1962 studied the rhizome rot of ginger and its control measures. Bal gopal, Devi, Indrasenan and Wilson (1975) studied the varietal reactions of ginger towards soft rot caused by P. aphanidermatum from Kerela. Bhagwati,(1960) studies soft rot of ginger and its control measures. Indrasenan, & Paily, (1973) have also studies P. aphinadermatum causing soft rot of ginger from Kerela.

Pythium species mainly infect juvenile or succulant tissues. Thus restricts their parasitism to seedlings or the feeder roots or root tips of older plants and to water fruits or stem tissue. They do not spread widely throughout host cells and are quickly followed by more aggressive or faster growing fungi with certain hosts such as grass, tomato transplants, peanuts and chrysanthemum. These fungi also attack stem and foliage of non seedling plants. They also cause fruit rots of crops such as beans squash and watermelon. Pythium also affects fruits, vegetables in storage and transit, bulb and tubers of horticulture plants before and after harvesting.

Pythium species commonly infect seed and the radicals causing seed rot and pre emergence damping off. The fungi also infect newly emerged seedlings at ground level causing them to collapse or topple over, a common symptom of post emergence damping off. The disease is most noticeable in nursery beds, green houses and row crops because symptoms develops suddenly, killing large number of seedling in local area.

Pythium species survive in soil by saprophyticgrowth and by resistant resting structures. They are not vigorous competitors.

The survival of P. ultimum sporangium in soil has been reported by Stanghellini & Hancock (1971).

PART I

SECTION B

PATHOGENICITY

CHAPTER V

INTRODUCTION

Pathogenicity experiments have been conducted by Buttler (1907), Buttler & Bisby (1931), Subramaniam (1919), Thomas (1938), Middleton (1945), Patel et.el.(1949), Bhagawat (1960) Sharma (1977), Sharma & Joshi (1977), Shahare and Asthana (1962), Haware and Joshi (1973,74) , Sharma, Joshi and Jain (1976) have made a significant study on the pathogenicity of P.aphanidermatum on ginger rhizome at Jabal pur, (M.P.).

These workers have also isolated P.aphanidermatum from the diseased ginger rhizomes and have confirmed that loss is caused by fungus to the ginger plant. These workers have also observed the same symptoms during pathogenicity of the host. Symptoms of the diseases have already been described in Section `A'.

Isolation experiments conducted by Sharma & Jain (1977) included isolation of pathogen from the (1) washings of rhizome (2) superficial peeling of surface sterilized rhizome with .1% HgCl2 (3) inner deep portion of the rhizome. These isolation were however done from stored rhizome and from fresh rhizome. The present author also succeeded in isolating the pathogen from surface sterilized rhizomes brought to the lab in sterilized petriplates from the fields. Inoculation studies were also done by the above workers. Indrasenan and Paily (1973) made inoculation experiments on germinating buds of ginger rhizomes and young ginger plants and on matured rhizomes. They also screened 21 varieties of ginger for their susceptibility to the disease. These were done with 48 hours Pythium cultures with and without injury. Pathogenicity and inoculation experiments were also conducted by Haware and Joshi (1973) in pots. In this, inoculation was done by using mycelial suspension of the test fungus in which freshly cut surface sterilized rhizomes were dipped and then planted in sterilized soil in pots. The pathogenicity was confirmed by these workers in the experiments. On the same lines the author has also planned the pathogenicity in culture tubes, sterilized and in unsterlised soil using mycelial inoculum ,diseased host baits and sand oat meal inoculum. The pathogen was grown on different media by various workers to study the morphological behaviour of Pythium .Among these are Mitra and Subramaniam(1928), Hickman (1944) Drechsler (1952-53),Singh and Srivatsava (1953), Emerson (1958) and Bineeta Sen and Srivatsava (1960). These workers mainly aimed their experiment to study the factor affecting zoospore production in Pythium aphanidermatum. The present author have also made a detail study using various culture media aiming to select the media suitable for the growth of the pathogen.

The genus Pythium is very sensitive towards temperature both for its growth and production of zoospores. Normally the fungus grow to an optimum level at a temperature 26 - 28°C. The zoospore production takes place in the morning at a still lower temperature . The studies of Cantrell and Dowler(1971) have also pointed to the same conclusion. Since disease incidence is closely related to the temperature the author studied the effect of temperature on the pathogen.

The effect of C:N ratio on the growth of Pythium species have been studied by Kraft and Erwin (1967) ,Bineeta Sen and Srivatsava (1968) used organic matter to change the C:N ratio and studied the effects on the zoospore production of P.aphanidermatum .Muralidharan and Raman Kutty (1975) studied the effect of Nitrogen on ginger production. Muralidaran, Nair and Balkrishnan(1973) studied the fertilizer requirement of ginger.

Ginger gave better yield when the fertilizer were added to the soil.Grover and Sidhu(1966) studied the effect of nitrogen sources on the growth of Pythium aphanidermatum .Saxena et.al(1952) made a study on sulphur and nitrogen requirement of the genus Pythium .

The above studies pointed the worker to study the effect of C:N ratio on the growth of the pathogen and thus was included in the present studies.

Similarly the age of culture and rhizome on the disease development have been studied by a number of workers.

Effect of age of culture on zoospore production have been done by Bineeta Sen and Srivatsava (1968).

Age of rhizome on the disease development have been similarly studied by Indrasenan and Paily (1973). The author too studied the effect of age of culture and rhizome on the disease development. Germination invivo of P.aphanidermatum zoospore sporangia has been described by Stanghellini and Burr (1973) .The above studies have been planned and conducted in the manner described in the chapter experimental and discussed and concluded in the preceding chapter.

CHAPTER VI

EXPERIMENTAL

Experiment (I)

Isolation of Pathogen from infected rhizomes brought from various ginger fields.

The plant suspected for disease were taken out alongwith the soil with the help of digging blades ,care was taken to avoid any injury to underground rhizome. The soil crumbs was broken gently so as to cause the least possible damage to the rhizome. The soil obtained was collected in poly bags for isolation of pathogens .

The diseased underground rhizomes suspected to be under different stages of infection were collected and brought to the laboratory in different sterilized petridishes for isolation of the pathogen.

For isolation the pathogen infected rhizome and other parts were washed with tap water to remove adherant soil and were then cut into pieces of 1.2 cm length ,surface sterilized by 0.1% mercuric chloride for 2-3 minutes and then washed repeatedly with sterilized water to remove disinfectant. Surface sterilized and washed pieces were plated in following medium and incubated oat 26 ^{0}C. for seven to ten days.

1. Potato Dextrose Agar
2. Potato Dextrose + Pimaracin and Vancomycin.

Experiment (II)

A. In-oculation Experiments

Pathogenicity Test

The pathogens was tested for pathogenicity on freshly developed ginger, rhizome obtained from local horticulture department and old matured rhizomes obtained from the local market. To confirm the pathogenicity, preliminary test was conducted in culture tube experiments.The final detailed pathogenisity test was done in soil and in jars.

1. Culture tube experiments.

Culture tube 20 X 3.5 cm size were used. Cotton plugs soaked with soil extract was placed in it. Two bits of rhizomes surface sterilized with 0.1% HgCl2 and washed repeatedly with sterilized water and placed in each tube.The in-oculum was added in the form of 8mm

agar disc cut away from the margin of an actively growing colony of the pathogen. The tubes were kept at 26°C.and observed periodically for five to eight days. The result obtained are tabulated in Table I

2. Experiment in soil

The two types of inocculum of the pathogen were used :

i.As Mycelial ino-culum and

ii.In the form of diseased host baites.

i. Preparation of mycelial in-oculum:

Sand was passed through a sieve of 4mm mash and was washed repeatedly to remove small sand particles,clay and other substances. After drying 100gm of sand,3gms of oat meal and 35 ml of water in 250ml concial flask were autoclaved for 45 minutes at 20 pound pressure. These were kept readily and ino-culated with the pathogen as and when required.The flask were ino-culated with a 8mm agar disc cut from the margin of the freshly growing colony of the pathogen. The flask were incubated for three weeks at 28 °C. and vigorously shaken so as to get a homogenous mixture.

ii. Preparation of Diseased Host Baites:

Rhizomes were repeatedly washed to remove aherant soil and surface sterilised in .1% HgCl2 for 7-8 minutes. Skin was peeled exceptionally and rhizome was then cut into small pieces. These were then again sterilised for 3-5 minutes then again repeatedly washed with sterilised water and they were then kept aceptically in sterilised flask containing 50ml. Potato Dextrose Agar medium inoculated 5days earlier with the pathogen and incubated at 28°C. The baites showed symptoms of rotting and were completely invaded by mycelium after 21 days. These were taken and used for inoculating the soil.

B. Glass Jar Experiments

These experiments were performed in sterilised and unsterilised soil for both the freshly developed and old mature ginger rhizome. All experiments were done in triplicates. Field soil was brought to the lab and dried & seived with 6mm mash to fill the experiment jar. Two control for each stage of ginger used were run side by side. Half of the jars were autoclaved at 20 pound pressure for 45 minutes, the remaining half, un-auto claved jars were kept to study the pathogenicity in unsterilised soil. 15% of sa- nd in-oculum was mixed thoroughly with the soil in each pot. Water equal to 50% of the moisture holding capacity of soil was added. Two surface sterilised rhizome were transfered in each experiment jars.

The jars were kept in glass house at 26 ± 2 °C and loss of water was made good -by adding water intermittently. The jars were observed regularly to note the development of disease symptoms. Control jars receiving sand only instead of inoculum were also observed and the observations made for 5 to 8 days after inoculation are given in table II.

Experiment III

Effect of different culture media on the growth of the pathogen

Studies were carried out to determine the influence of different culture media upon the growth of pathogen. The experiments were conducted on both broth and solid media. In broth media still cultures of the pathogen were grown in 100 ml conical flask containing about 35ml of the broth medium. The inoculum in each case consisted of a disc of 8mm diameter, cut from the margin of the fresh growing colony of the pathogen on PDA. After 10 days the mycelial mat was collected and separated by filteration under suction in buchners funnel on previously weighed Wattman No.42 filter paper.Mycelial mat was washed with distilled water. The filter paper with mycelial mat was placed in the oven at 80°C. Dry weight of the mycelium was then calculated after reweighing the filter paper with their content. Similarly radial growth of the pathogen was examined on solidified agar. Media was poured in petri plates. After the media solidified the plates were inoculated with the pathogen after 0 hrs, 24 hrs, & 48 hrs . The inoculum consisted of single disc of 8 mm size cut from the margin of freshly grown colony and placed in the centre of the petri plates. The radial growth was recorded after every 24 to 72 hrs, and growth ploted against time. The following media were used for the purpose:

1. Potato Dextrose Agar
2. Czapecks Dox Agar
3. Richards Medium
4. Peptone Dextrose Agar and
5. Glucose Asparagin.

1. PDA

Agar	: 17 gms
Potato peeled & sliced	: 200 gms
Dextrose	: 20 gms
Water	: 1000 ml.

Potato peeled and cooked for one hour or 40 minutes in autoclave in 500ml of water at the same time agar is melted in 500ml of water. The potato juice is decanted in melted agar and volume adjusted with water. Dextrose is then added

2. Czapecks Dox Agar:

Agar	: 15 gms
$NaNO_3$	: 2 gms
K_2HPO_4	: 1 gm
$MgSO_4.7H_2O$	: .50 gms
KCl	: .50 gms
$FeSO_4.7H_2O$	: 0.01 gms
Sucrose	: 30 gms
Distilled water	: 1000 ml

Sucrose is added prior to the final sterilisation. Czapecks contains 2 gms NaNO3 per litre. 1 gms yeast extract will be added.

3. Richard's Medium:

MgSO4.$7H_2O$	: .25 gms
KH_2PO_4	: 5 gms
KNO_3	: 10 gms
Sucrose	: 50 gms
Potato Starch	: 10 gms.
$FeCI_3$	: Traces
Distilled water	: 1000 ml

4. Peptone Dextrose Agar:

Agar	: 20 gms
KH_2PO_4	: 1 gm
$MgSO_4.7H_2O$	: 0.5 gms
Peptone	: 5 gms

Dextrose : 10 gms

Distilled water : 1000 ml

5. Glucose Asparagin:

Glucose : 10 gms

Asparagin : 5 gms

KH_2PO_4 : 1 gm

$MgSO_4.7H_4O$: 0.50 gm

Distilled Water : 1000 ml

Experiment (IV):

1. Effect of change in C:N ratio on the growth of pathogen:

The pathogen was grown on petri plates on Czapecks Dox medium.In one set of experiments the concentration of nitrogen was kept constant and the amount of carbon was changed by varying the amount of Sucrose used i.e., 15 gms, 30 gms and 45 gms / Litres.While in another set concentration of sucrose was kept constant and the quantity of nitrate was changed i.e., 1 gm, 2 gms, and 3 gms/Litreof medium preparation. The medium was allowed to solidify for 24 hrs.

Inoculation of the pathogen was done with a 8mm disc cut away from the tip of the newly grown colony of the pathogen . Radial growth was recorded after every 24 hrs to 72 hrs and plotted against time. The data are given in Table V,Fig.VI.

5. Experiment (V):

Effect of temperature on growth of pathogen:

For the experiment sterilised petri dishes were poured with Potato Dextrose Agar medium and left overnight so that surface water could evaporate. Then each plate was inoculated in the centre with 8mm disc cut away from the actively growing colony of the pathogen. The inoculated disc were incubated at 15^0C , 25^0C, 35^0C, 45^0C. Radial growth of the pathogen was recorded after every 24 hrs upto 72 hrs. The growth recorded is given in the Table VI.Fig.V.

6. Experiment (VI):

The effect of age of culture on the disease development:

The experiment will be performed in the same way as the culture tube experiment with the variation that the cotton plugs will be soaked in Czapecks Dox medium and the tubes sterilised. The tubes shall be incubated. The surface sterilised rhizomes were introduced to the tubes after the interval of 3 days, 5 days and 8 days. The disease incidence was recorded in Table VII, after the inoculated rhizomes were incubated at 26 ^{0}C, for 5-8 days. The rhizomes were given 2 or 3 pricks to get quick results.

7. Experiment (VII):

Effect of age of rhizomes on the disease development:

For the experiment no separate expriment was conducted as the effect of the rhizome was observed from the earlier experiment on pathogenicity. The author had used in the pathogenicity experiment rhizome of two different age group. One was the freshly developed rhizome and the other old and mature rhizome. The observation recorded are shown in table I, II and VII. After considering these results and those of Exp VI table VII the necessity of as fresh experiment wasnot felt.

CHAPTER VII

RESULTS AND OBSERVATIONS

Experiment 1

Isolation of pathogen

The suspected rhizome for disease were brought in sterilised petridishes from the field to the laboratory. The pathogen was isolated from those infected rhizomes on PDA and PDA with Pimaracin and Vancomycin. The growth appeared as a white cotton mass of entangled hyphae of the pathogen. The isolated pathogen was found to be P. aphanidermatum . The identification was done with the help of monograph and other available literature refered in the previous chapter and Bibiliography.

Experiment 2

Inoculation experiments

a) Culture Tube Experiments

Sterilised tubes 20 x 3.5cm size were used in this experiment and pathogenicity was tested under sterilised conditions. The results obtained are given in the table I.

The observationtable shows that the freshly developed rhizomes are more susceptible to infection as compared to the old and mature rhizomes i.e., 80% and 15% respectively after 5days of inoculation. However , the mature rhizomes were also infected almost with the same frequency as the rhizomes were more exposed to pathogen for a long period of time under sterilised condition, i.e, after 8days of inoculation . It appears that the old rhizome must have developed some substance which must be having protective function at least for some period of time. The rotting under control conditions must be due to presence of soil extract in abundance, or the rhizome used might not be free from pathogen.

b) Glass Jar Experiment

After testing the pathogenicity in the culture tubes experiment, confirmation was done by testing the pathogenicity in the soil under sterilized condition and unsterilized condition using glass jars.

For the experiments both sand oat meal inoculum and diseased host baites were used for inoculating the soil. The datas obtained are given in the table II. From the observations pathogenicity was confirmed. Again it was noticed that the freshly developed rhizome were more susceptible for disease development as compared to the old andmature

rhizomes.The disease was more underthesterilised condition while under unsterilised condition it was less. This must be on account of the resistance faced by the pathogen from other soil microbes present in unsterilised soil. While under sterilised condition the pathogen grew freely and unchecked due to abundance of moisture under their disposal.

Experiment 3

Effect of Different Culture Media.

For the experiment four different media were taken namely Potato Dextrose Agar, Peptone Dextrose Agar, CzapecksDox, and Glucose Asparagin Media. The experiments were conducted on both broth and solid medium. In broth media the culture of the pathogen were grown in 100ml sterilised conical flasks.After 10 days of growth the mycelial mat was collected on Wattman filter paper No.42,and dry weight noted can be seen in Table III. From the table it will be evident that the maximum mycelial mat was found on Potato Dextrose ,medium folowed by Czapecks Dox and Peptone Dextrose medium i.e 295,130,90 mg.respectively. The minimum mycelial mat was found in Glucose Asparagin i.e.5mg only.

Similarly on solidified agar the radial growth of the pathogen was observed.The datas are given in Table IV.Fig. I - III. The medium was poured in sterilised petriplates and after solidification pathogen was inoculated in the centre of the petridish after 0 hr, 24 hrs and 48 hrs. The radial growth was recorded after every 24 hrs.to 72 hrs. From the table,it can be observed that Potato Dextrose Agar was the most suitable medium for the growth of the pathogen followed by Czapecks Dox. Growth of the pathogen was fastest when inoculated after 0 hrs.and slowed down as the inoculation was delayed and done after 24 hrs.and 48 hrs. This shows that drier the medium, less is the growth i.e the moisture content has an important role for the growth of P.aphanidermatum .

Experiment (IV)

Effect of C:N Ratio on the growth of the pathogen

The effect of C:N ratio on growth was tested on solidified Czapecks Dox Medium in sterilised petriplates.In this medium in one set of experiments the concentration of $NaNO_3$ was kept constant and the quantity of sucrose used was changed i.e 15gms,30gms,and 45gms /litre.,respectively. In another set of experiment the concentration of sucrose was kept constant and concentration of nitrate was changed i.e 1gm,2gm.,3gm.,respectively. The data recorded are tabulated in Table V Fig IV;.Maximum growth was recorded in the medium when $NaNO_3$ was taken as 2gms /litre and sucrose 30gms/litre.The rate of growth was almost the

same when both the quantities were reduced to 50 %. With the increase in NaNO3the growth decreased.

Experiment V

Effect of temperature on growth of pathogen

The pathogen was inoculated on the previousely sterilised and solidified Potato Dextrose Agar petriplates.Incubation was done in triplicate at 15^0 C.,25^0 C, 35^0 C, 45^0 C. The radial growth of the pathogen at different temperature was noted after every 24 hrs to 72 hrs and given in Table VI and Fig V.

Considering the datas given in the table it is evident that temperature affects the growth of the pathogen.The optimum temperature for the growth of the pathogen was found to be 25^{0}C where the plate were fully grown by the pathogen in 48 hrs,while at 35^0 C. the pathogen could develop the diameter of 7.3cm only. The same was the condition at 15^0 C but at 45^0 C. the mycelium could not give any significant growth. The studies conducted by Cantrell and Dowler in 1971, revealed that the optimum temperature for mycelial production was lower in liquid medium than Agar medium.

Experiment VI

Effect of age of the culture on disease development

To study the effect of the culture on disease development the culture were raised for 3,5,and 8days respectively.The disease incidence was recorded in Table VII.The data obtained showed that3 days old culture of the pathogen was most effective and caused 75% loss to the freshly developed rhizome and 50 % loss to the old and mature rhizome.As the culture grew older the capacity to produce disease lowered. Minimum disease incidence was recorded with 8 days old culture i.e. 30% on freshly developed rhizome and 20% on old and mature rhizome.

Experiment VII

Effect of age of Rhizome on the disease development

To see the effect of age of rhizome on the disease development the rhizomes of two different age group i.e one freshly developed rhizome while the other old and mature rhizome were used.The observations are recorded in Table I,II and VII. The observation tables show that freshly developed rhizomes were more susceptible to infection as compared to old and mature rhizomes. However,it was found that if the pathogen is kept in contact for a long time with old and mature rhizome ,they get involved in pathogenesis.The delay in the disease

development on the old and mature rhizome must be the accumulation of some substances which might be having an inhibitory effect on the pathogen.During the studies on isolation the author has visited several fields and there also it was found that among the infected rhizome collected most of them belong to the freshly developed group.Thus it cannot be said that Pythium aphanidermatum affects the ginger rhizome in the early stage of development while during the later stage the disease incidence is very less.

TABLE I

CULTURE TUBE EXPERIMENT

SNO	RHIZOME USED	NO. OF TUBES INOCULATED	% INFECTION AFTER DAYS	
			5 DAYS	10 DAYS
1.	Freshly developed Rhizome	20	80%	100%
2.	Old & matured Rhizome	20	15%	80%
3.	Control	10	NIL	10%

TABLE II

GLASS JAR EXPERIMENT

Condition of Rhizome	STERILIZED SOIL				UN STERILIZED SOIL			
	No. of Experiment jars	No. of Rhizome	Dev. of disease	% of disease development	No. of Experiment jars	No. of Rhizome	Dev. of disease	% of disease development
Freshly developed Rhizome	3JARS inoculated with pathogen	6	6	100%	3JARS inoculated with pathogen	6	5	83.3%
	2JARS Control	4	NIL	0%	2JARS Control	4	nil	0%
Old and Mature rhizomes	3JARS inoculated with pathogen	6	4	66.6%	3JARS inoculated with pathogen	6	3	50%
	2JARS control	4	NIL	0%	2JARS control	4	NIL	0%

TABLE III

EFFECT OF DIFFERENT BROTH CULTURE MEDIA ON THE GROWTH OF P.aphanidermatum

	CULTURE MEDIA	WEIGHT OF PAPER	WEIGHT OF PAPER MYCELIUM	WEIGHT OF MYCELIUM MAT.
1.	Potato Dextrose	865mg	1160mg	295mg
2.	Glucose Asparagin	810mg	815mg	5mg
3.	Peptone Dextrose	855mg	945mg	90mg
4.	Czapecks dox	860mg	990mg	130mg

Data recorded from 10 day old culture medium

TABLE IV

EFFECT OF DIFFERENT AGAR MEDIA ON THE RADIAL GROWTH OF P.aphanidermatum

CULTURE MEDIA	INOCULATION After 0 hrs			INOCULATION After 24hrs			Inoculation After 48 hrs		
	GROWTH IN HRS. 24	48	72	24	GROWTH IN HRS. 48	72	24	GROWTH IN HRS. 48	72
1.Peptone Dextrose	4.5cm	8.0cm	full	3.25cm	6.5cm	full	3.1cm	5.2cm	7.6cm
2.Potato Dextrose	9.0cm	full	full	4.6cm	7.5cm	full	4.6cm	6.5cm	8.5cm
3.Czapecks Dox	8.1cm	full	full	4.5cm	7.5cm	full	4.2cm	6.4cm	8.0cm
4.Richards medium	3.8cm	7.0cm	full	3.0cm	5.5cm	full	2.8cm	4.7cm	6.3cm

TABLE V

EFFECT OF C:N RATIO ON THE GROWTH OF P.zeheniderma tum

Sno.	Culture media	concent. of NaNO3	concent. of carbon source	Growth in Hours 24	48	72
1.	Czapecks Dox	3 gms	15gms	1.7cm	2.8cm	3.2cm
2.	Czapecks Dox	2gms	15gms	2.00cm	3.5cm	6.0cm
3.	Czapecks Dox	1gm	15gm	4.1cm	6.7cm	full
4.	Czapecks Dox	2gms	30gm	4.5cm	7.2cm	full
5.	Czapecks Dox	2gms	45gms	3.7cm	5.8cms	8.2cms

TABLE VI

EFFECT OF TEMPERATURE ON THE GROWTH OF P.sphanidermatum

MEDIUM	INCUBATION TEMPERATURE	RADIAL GROWTH OF THE PATHOGEN AFTER HRS		
		24	48	72
PDA	15 DEGREE CENTIGRADE	4 cms	5 cms	6 cms
PDA	25 DEGREE CENTIGRADE	4.4 cms	7.5 cms	full
PDA	35 DEGREE CENTRIGRADE	4.6 cms	6.5 cms	7.3cms
PDA	45 DEGREE CENTIGRADE	1.5 cms	1.6 cms	1.6 cms

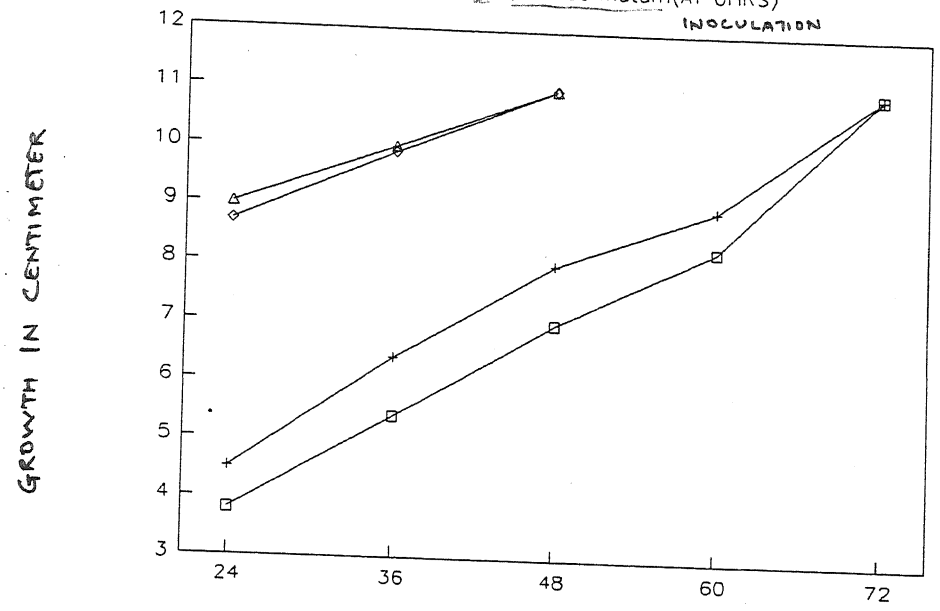
TABLE VII

EFFECT OF AGE ON CULTURE AND RHIZOME ON DISEASE INCIDENCE

Age of the Culture	Freshly Developed rhizome	Old and matured rhizome
3 DAYS	75%	50%
5 DAYS	53%	36%
8 DAYS	30%	20%

EFFECT OF DIFF.AGAR MEDIA ON RADIAL

GROWTH OF P.aphanidermatum(AT 0HRS)



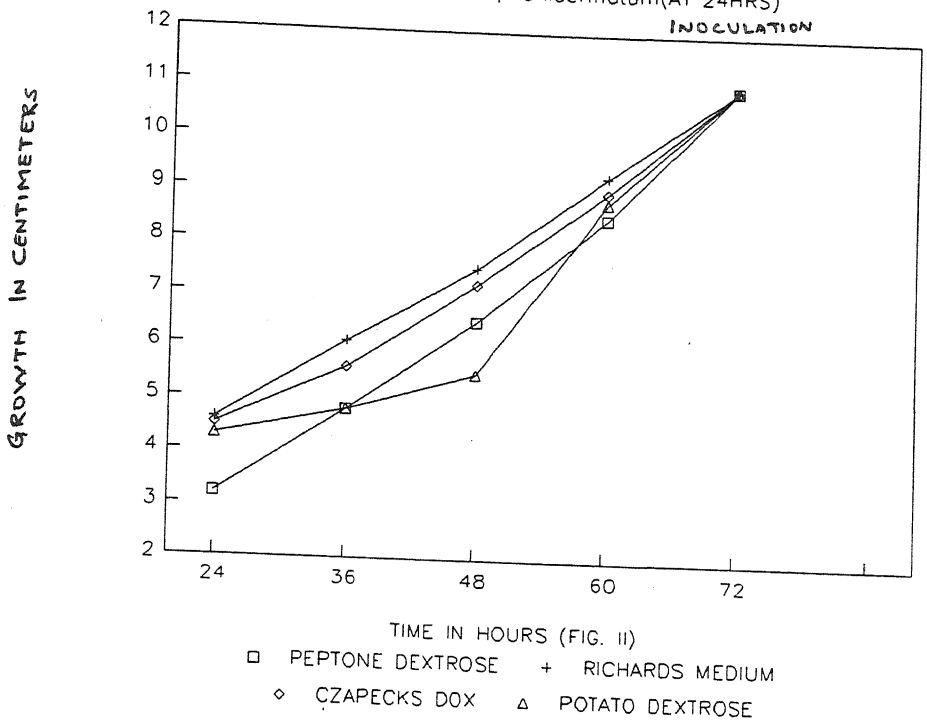
TIME IN HOURS (FIG. 1)

□ RICHARDS MEDIUM + PEPTONE DEXTROSE

◇ CZAPECKS DOX △ POTATO DEXTROSE

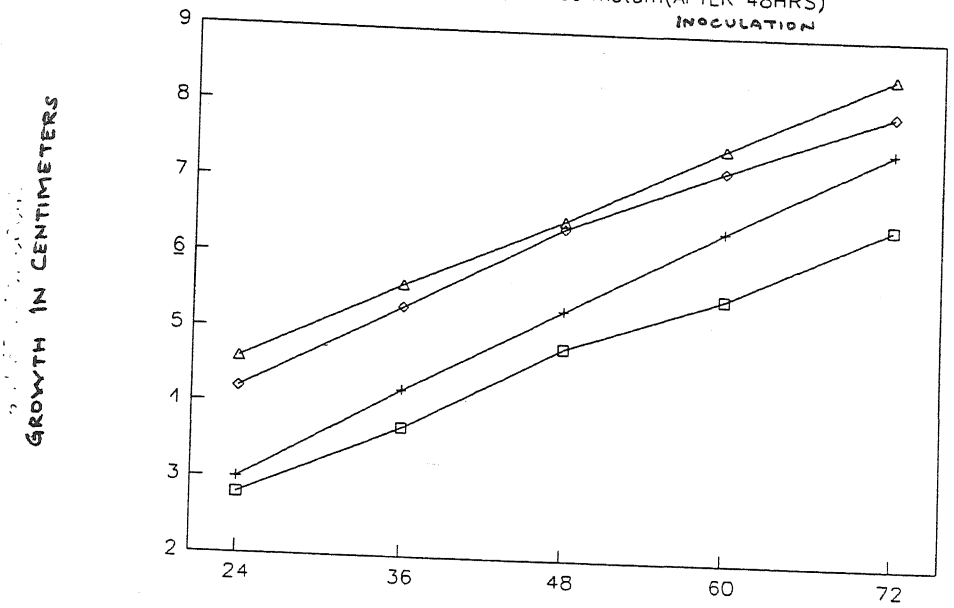
EFFECT OF DIFF.AGAR MEDIA ON RADIAL

GROWTH OF P.aphanidermatum(AT 24HRS)



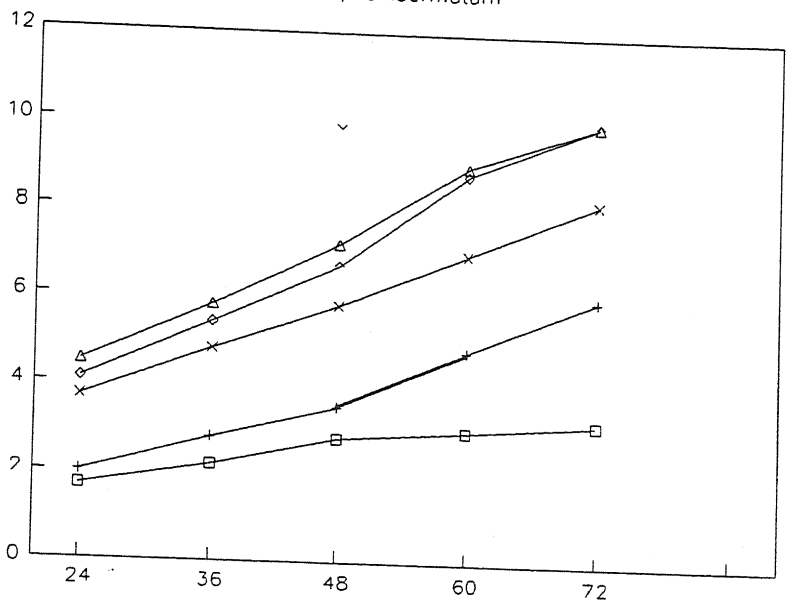
EFFECT OF DIFF.AGAR MEDIA ON RADIAL

GROWTH OF P.aphanidermatum(AFTER 48HRS)



EFFECT OF C:N RATIO ON GROWTH OF

P.aphanidermatum

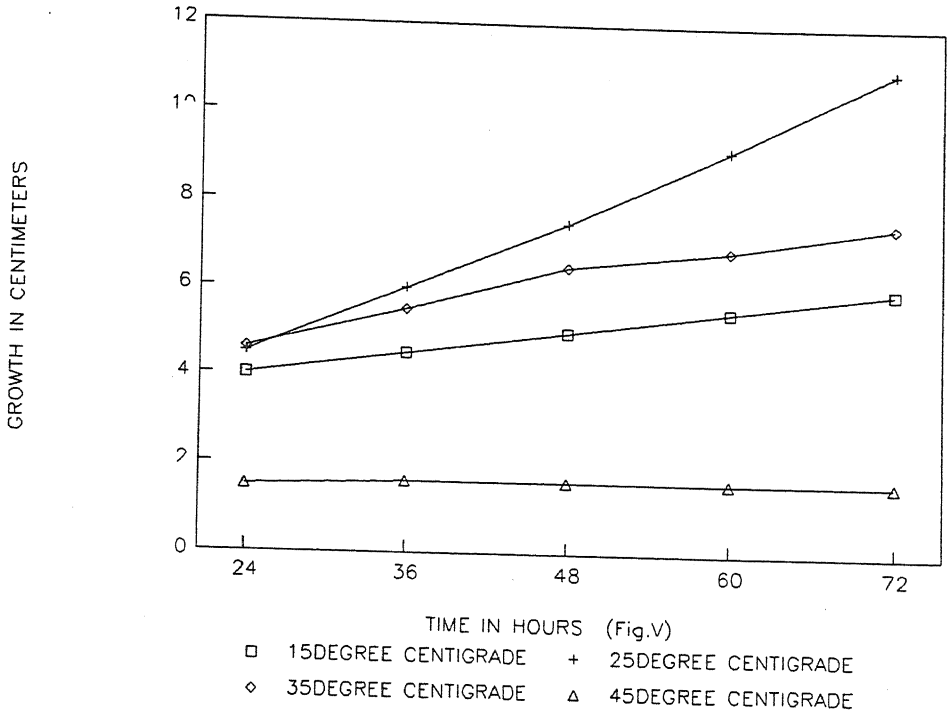


TIME IN HOURS (FIG. IV)

□ 3 GMS NaNO3/15GMS C + 2GMS NaNO3/15GMS C

◇ 1GM NaNO3/15GMS C △ 2GMS NaNO3/30GMS C × 2GMS NaNO3/45GMS C

EFFECT OF TEMPERATURE ON THE GROWTH OF P. aphanidermatum



CHAPTER VIII

DISCUSSION AND CONCLUSION

Soft rot of ginger is though an old disease and is reported from various places and has also been studied by a number of workers. From the earliest report of Buttler (1907), Subramaniam (1919 till Haware and Joshi (1972), Indrasenan and Paily (1973) has been worked out in South India and Madhya Pradesh). But it was recently very prominent in the Bundelkhand region. The loss has been found to be enormous and is therefore considered to be important for the present study. Baruasagar the main centre of ginger cultivation is worst affected and has been replaced by the farmers with some other crops.

During the isolation studies the author isolated P.aphanidermatum (Edson) Fitz from infected rhizomes.During the field study the symptoms were observed on the leaves which show the illness and yellowing particularly at the leaf tip which gradually spreads down leaf blade. The dead leaves hang down and the basal portion at the place of attachment showed paleness.The rhizomes were observed with watery mass and destruction of paremchymatous tissue. The roots also start rotting and softening.Rhizomes brought from storage region also showed similar symptoms. Young rhizomes were found to be worst affected and adult plants also failed to produce rhizomes. The disease noticed by the author was more abundand at palaces where water lodged conditions were more and soil was rich in clay fraction.Rajan and Singh(1973) found similar observations where they reported that increase in sand fraction supressed the growth of Pythium.This might be the result of water logged conditions in clay which facilitates the growth of Pythium. Garett (1956) made similar observations light structured soil being poor in nutrient capacity and acidic reaction donot favour development of soil bound diseases. P.aphanidermatum from soft rot of ginger has been isolated by Rajan and Singh (1974)on Peptone Dextrose, Rose Bengal,with Piramycin and Dichrysticin.The author however isolated pathogen on Potato Dextrose Agar, PDA with Piramycin and Vancomycin.The isolation was done from infected rhizomes ,superficial peeling and deeper tissues after surface sterilising them with .1% HgCl2. Sharma and Jain (1977) isolated Pythium deliense from washing of rhizome,superficial peeling and deeper tissues on PDA.

P.aphanidermatum ,the casual organism for the soft rot of ginger has also been isolated by Shahare and Asthana (1962) from Chindwara,Madyapradesh,Park (1934-41)isolated P.aphanidermatum from Ceylon and Surat respectively and Indrasenan and Paily (1973) from Kerala.

The symptoms of the disease as studied by the author are in confirmity with

Chattopadhyaya (1967).In the pathogenicity experiments,the author performed tests in culture tubes and in soil under sterilized as well as in unsterilized conditions.Results of the culture tube experiments are given in the Table I.The observations show that freshly developed rhizomes are more susceptible as compared to old rhizomes. After 5 days of isolation 80% disease incidence was noted on fresh rhizomes and 15% on old rhizomes.However the percentage increased after 3 days of incubation. Glass jar experiments (Table II) having sterilised and unsterilized soil confirmed the results of the culture tube. The disease incidence was more under sterilized conditions as compared to the unsterilized condition.This might be obviously due to the presence of other microbes under unsterilzed conditions which might be antagonistic to the pathogen or might be checking the growth of pathogen by competeting for space and nutrients.The author observed that the infection was better with pricked rhizome as compared to the unpricked ones.These observations are similar to those of Indrasenan and Paily (1973).Pathogenicity of the P.aphanidermatum causing rhizome rot of ginger has also been confirmed by Mitra and Subramaniam (1928),Shahare and Asthana (1962).For the further studies on the pathogen ,its growth on different culture medium was studied.Bineeta Sen and Srivastava (1968)used ten different culture medium to study the effect of zoospore production by P.aphanidermatum. The author used five different medium Potato Dextrose,Glucose Asparagin,Peptone Dextrose,Czapecks Dox and Richards medium.The experiments were conducted on broth and on solid medium. In broth dry weight of the mycelial mat was obtained after ten days of incuabtion and considered the criteria for the growth of the pathogen.The results obtained are given in Table III .Dry weight of the mycelium on PDA ,Czapecks Dox ,Peptone Dextrose and Glucose Asparagin were 295,130,90 and 5 mg respectively.

On solid media the radial growth of the pathogen was recorded after every 24 hrs. upto 72 hrs.Solidified Agar medium was inoculated after 0hrs,24hrs.,48hrs. respectively.The pathogen grew fastest in plates inoculated after 0 hrs. then slowed down when inoculated after 24 and 48 hrs. respectively.,in all the mediums used.Table IV Fig I,II,and III reflect the pattern of growth followed by the pathogen at 0 hrs.The pathogen filled the entire plate by its radial growth,in 48 hrs..on PDA and Czapecks Dox.While on Peptone Dextrose and Richards medium.This growth could only be observed after 72 hrs. At 24 hrs the PDA petridishes were completely full after 72 hrs. Plates which were used after 48 hrs of pouring the pathogens could not cover the entire plate even after 72 hrs of incubation. This growth pattern also shows that the pathogen could not grow better in the medium where nitrogen is present.PDA is a semi synthetic medium where the sole contents are potato extracts and dextrose.Thus gave the best report.This observation of the author is similar to Bineeta Sen and Srivastava

(1968) who found better zoospore production in the medium having Corn meal or Oat meal.

The effect of C:N ratio on the growth of P.aphanidermatum was felt by the author in the experiment described above.

To have a better understanding the author conducted an experiment using Czapecks Dox medium.In one set of experiment NaNO3 was kept constant and,Sucrose concentration was changed i.e15 gms,30 gms and 45gms respectively. In another set using the same medium sucrose concentration was kept constant and,the quantity of NaNO3 was changed i.e 1gm,2gm,3gms respectively.From the data recorded in the Table V Fig IV it can be observed that the best radial growth was obtained in medium using 2gms NaNO3 and 30 gms Sucrose.When the concentration of NaNO3was increased or Sucrose was decreased the growth decreased.Influence of nitrogen compounds on the growth of Pythiumspecies have been studied by Agnihotri and Vaartaja(1967), Bineeta Sen and Srivastava (1968) also could not find zoospore production from P.aphanidermatum when aqueous NH4NO3 solution(2%) or an aqueous Peptone solution(.25%) was used. Nitrogen not only influences Pythium growth in medium but also when applied to soil in the form of fertilizers reduced disease incidence. Rajan and Singh (1974) made the similar observation when they found reduction in isolation of Pythium from soils with 30ppm nitrogen. Vanterpool (1940), Hooker (1953), Agnihotri and Vaartaja(1967) and Kauraw(1970) have also found that soil fertilizers having Nitrogen reduces the severity of the disease caused by Pythium. This suggest that higer level of nitrogen is toxic to P.aphanidermatum. Thus this finding of the author is similar to those of the above workers. Ginger yield is increased with the addition of nitrogen at the rate of 30kgs/ hectare[Murlidaran and Raman Kutty (1975)]. The observation is again an indication to the fact that under the above concentration Pythium is not able to grow.

Pathogens behaviour towards the incubation temperature of 15^{0}C 25 ^{0}C, 35 ^{0}C and 45 ^{0}C was studied by the author by growing the pathogen and observing the radial growth after every 24hrs to 72 hrs. The result obtained in table VI and fig.V show sthat the growth was best at 25 ^{0}C followed by 35 ^{0}C , 15 ^{0}C and 45 ^{0}C This observation of the author is in contrast to the one observed by Middleton (1943) who found optimum temperature for the growth of Pythium to be about 34^0 C and maximum around 40^0 C.

Cantrell and Dowler (1971) found that optimum temperature for Pythium was low on liquid medium than on Agar. The temperature response of Pythium towards radial growth indicates to the fact observed by the author that soft rot of ginger occured in the fields at the time when the atomspheric temperature was around 25^{0}C .

Age of the culture of pathogen have also been found to be an important factor in the

disease incidence. During cultural experiments the author raised the culture and inoculation was done on the rhizome after 3,5 and 8days respectively. The datas obtained in table VII show that 3 days old culture was most effective and caused 75% loss to the freshly developed rhizome and 50% to the old and matured rhizome . The disease incidence lowered as they grew old. With eight days old culture only 30% loss on freshly developed rhizome and 20% loss on old and mature rhizome was observed. Similar observation was recorded by Indrasenan and Paily (1973) where they found that 2 days old culture develop maximum infection. Bineeta Sen & Srivastava (1968) also found maximum zoospore production in 48 hours old culture and the capacity to produce zoospore gradually decreased with the increase of age of the culture. The age of rhizome is another factor responsible for the pathogenesis. This will be evident from the pathogenicity experiments conducted in culture tubes and glass jars already described. The observation recorded in table I,II VI clearly showed that freshly developed rhizomes and buds are more susceptible to infection as compared to old and matured rhizome. This fact was also noticed during field observations and isolation studies. In conclusion it can be said that P.aphanidermatum is more severe during the early stage of rhizome development. Similar observation were recorded by Inderasenan and Paily (1973). They studied the disease incidence on 4, 6, 8, 10 and 12 weeks old plant. % infection was 86, 70, 56, 36 and 30% respectively. Symptoms expression was also quicker in 4,6,and 8 weeks old plants. Mature plants and rhizomes where not easily infected. These obsevations confirm the observations of the present author.

PART II
ENZYMOLOGICAL STUDIES

CHAPTER IX

INTRODUCTION

Cell wall of higher plants contain large amount of cellulosic and pectic substances in their cell wall and middle lamella. The degradation of cellulosic and pectic constituents of cell walls and middle lamella occurs invariably in several types of diseases and has been reported for soft rot, dry rot, storage rot, wilts, blights etc. The degradation factor make the involvement of cellulolytic and pectic enzymes imperative in pathogenisis and thus leads to the loss of coherence between the cells. Further investigations in pathogenisis have shown that these enzymes are a constant feature in host pathogen interaction. However, non pathogenic fungi and bacteria have been found to produce pectolytic and cellulolytic enzymes in culture. Hence, these aspects show that there is no obligatory relationship of a micro organism in its ability to produce pectolytic and cellulolytic enzymes and the pathogenic capabilities but, they are certainly involved during pathogenisis in some way or the other. Pectic and cellulolytic enzyme production is one of the several properties of pathogen. They are therefore regarded as the complex of factors involved in pathogenisis. Therefore in this part both pectic and cellulolytic enzymes are studies in separate sections.

SECTION 'A': - PECTOLYTIC ENZYMES

In this section invivo and invitro production of various pectolytic enzymes by P.aphanidermatum were studies and described in the following manner :

Sub-section 'a':

Invivo studies of Pectic enzymes.

Sub-section 'b': Invitro studies of Pectic enzymes under various cultural conditions.

Sub-section 'c':

Invitro studies of Pectic enzymes in presence of growth Regulators and Fungicides.

SECTION 'B': - CELLULOLYTIC ENZYMES

In this section in vivo and in vitro studies of cellulolytic enzymes have been described in the following lines:

Sub-section 'a':

In vivo studies of cellulolytic enzymes

Sub-section 'b':

In vitro studies of cellulolytic enzymes under various cultural conditions.

Sub-section 'c':

In vitro studies of cellulolytic enzymes in presence of Growth Regulators and Fungicides.

PART II

SECTION 'A'

CHAPTER X

INTRODUCTION

Pectic substances are a class of carbohydrates having high polymerisation of certain organic acid. They are made up of linear polymeric chains of Degalactronic acid & Y 1-4 glycosidic linkage, Esterified with methyl groups or linked with other carboxyl groups via Calcium or Magnesium salt bridges. Pectic acid forms salts with various cations to form pectate and polypectate. They are the carboxyl chains not esterified. Pectic substances free from methyl groups is called "Pectic acid" When small portions of carboxyl groups are esterified which is less than 75%, the substance is called as "Pectenic acid". When relatively high proportion i.e., more than 75% of the carboxyl groups are esterified the substance is called "Pectin". The water insoluble parent substances in the primary wall are collectively referred as Protopectin. This is soluble in dilute acids and yield Pectinic Acid. The insolubility of Protopectinmay be due to its existance as a polygalacturonide.

The concept that pectic enzymes are involved in pathological manifestation induced in plant tissue by biotic agents was spowned by the studies of De Bary (1886) substantiated by the researches of Jones (1901) and of Brown (1915). Pectolytic enzyme obviously play a greater role in tissue disintegration. Pectolytic enzyme activity of root rot and damping off pathogen is involved in pathogenesis Hancock et.al.(1966), Bateman & Miller (1966), Mellano et.al.(1970). The importance of pectic enzymes in soft rot disease caused by P. aphanidermatum has been studied by Winstead & Mc Combs (1961). Physiology of parasitism of the pathogen has also been worked out by Indrasenan & Paily (1982) on soft rot of ginger. Successful penetration and establishment by the pathogen is accomplished by the elaboration of pectic enzymes in the cucumber host.

Conclusively , Pectic substances are high molecular acid polysaccharides. Pectic material constitute some of the more abundant polysaccharide in cell walls of higher plants and they are regarded generally as the principle constituents of the inter cellular cement or middle lamellar structure in plant tissue. Pectic substances are composed primarily of linear polymeric chain of D-galacturonic acid linked as $\alpha_{1,4}$ glycoside and containing carboxyl groups either not esterified (pectic acid) or esterified to different degree with methanol (pectinic acid and pectin).Jansen (1951) have found that there is a combined action of PG & PE during the maceration of Potato. Ayres et.al (1952) have given the influence of cultural condition on Pectolytic enzyme production "in vitro" Wood & Gupta (1958) studied the physiology and parasitism of P. debaryanum Gupta (1956) has also studies the cultural

conditions and their effect on the secretion of pectic enzymes of the above pathogen. He found that glucose, fructose and mannose were equally suitable for growth and enzyme production.

The occurrence of such a wide range of pectic substances involved were complex of pectolytic enzymes. The pectic enzymes involved are generally characterised as Protopectinase, Pectin Methyl Esterase, (PME), Pectic Methyl Galactronase (PMG), Poly Galactronase (PG), and Transeliminases or lyases depending upon

the nature of substrate undergoing enzyme action. The specific detail of action of these enzymes are being described below:

On the whole all the above pectic enzymes may be classified as

1. Protopectinases (macerating enzymes)
2. Pectin Methyl Esterases.
3. Chain splitting enzyme -

(i) Glycosidases (acting at low pH)

a. Poly Methyl Galactronase (PMG)

(Substrate Pectin)

b. Poly Galactonase (PG)

(Substrate Sodium Poly Pectate)

ii) Trans Eliminases or Lyases (acting at high pH)

a. Pectin Methyl Transeliminase (PMTE)

(Substrate Pectin)

b. Polygalactronase - Transeliminase (PGTE)

(Substrate Sodium Polypectate).

Production of Pectic enzyme invivo was studied in healthy as well as on diseased tissue of the host by Indrasenan & Paily (1982).

PROTOPECTINASE

Insoluble pectic substances are known as Protopectin and are mainly present in primary cell wall Protopectinase are the enzymes that converts protopectin into a soluble product. F.R. Davsion & J.J. Willaman (1927). Protopectinase assessed in terms of time taken (reaction

time) for disc of turgid potato tuber .5 mm thick and 8mm in diameter to loss of coherence after immersion in the test solution.

Macerating activity depends upon various factors like concentration, age of culture etc. Gupta (1956) studied the maceration of potato tissue by culture filtrates of P. aphanidermatum. Wood and Gupta (1958) has studied physiology of parasitism of P. debaryanum. Macerating enzymes activity have been assayed by the method adopted by Brown (1915) & Mellano et al (1970). Wood & Gupta (1958) observed that parenchyma of potato tuber was macerated by the culture filtrate of P. debaryanum. Bateman (1963) was of the view that macerating activity was not

the property of only one enzyme . Indrasenan and Paily (1982) found that P. aphanidermatum produce large quantities of these enzymes in young cultures, but these were incapable of macerating susceptible tissue. Winstead & Mc Combs (1961) were also of the same conclusion. Charvarty & Srivastava (1964) found free maceration of potato tissue by young culture filtrates of P. aphanidermatum..

PECTIN METHYL ESTERASES

SYNONYMS : Pectates,PectinMethoxylase,Pectinde Methoxylases,

Pectin esterases

Enzymes of this group remove the methoxyl group of pectic or pectinic acids to yield pectic acids. PME of fungi generally have a lower optimum pH for activity and are less influenced by the presence of salts in the reaction mixtures.

The action of pectin methyl estrase on pectin increases the rate at which the x-1,4 bonds of a pectic substances are cleared by those enzymes that are more reactive with pectic substances. PE's is used to describe the enzyme that de esterifies pectin. It catalyzes the hydrolysis of the ester bonds of pectic substances to yield methanol and pectic or pectinic acids. The name pectate was given by Fremmy (1840).

The action of these enzymes is assayed by measuring the increase in - COOH groups, the increase in CH3OH, the increase in sensitivity towards electrolyte (coagulation, gelation) decrease in viscosity etc., PEs are highly specific enzyme soponify almost exclusively the methyl ester groups of pectic substances.

Denel & Stutz (1958) are of the view that they are also found in culture fluids and regarded them to be adaptive (inducible) enzyme.

PME activity of P. aphanidermatum was not observed in vitro after 4 days of incubation

in different media, by Indrasenan & Paily (1982).

Charvarty & Srivastava (1964) has stated that PME has very significant role in maceration of the host cell by P. aphanidermatum. Winstead and Mc Combs (1961) has also come to the same conclusion.

Muthuswamy (1972) could not detect PME activity in vitroin infected tissues by P. aphanidermatum.

Winstead & Mc Combs (1961) found that there was no PME in the culture . Filtrates from diseased cucumber fruits contained PME. Extracts of healthy cucumber also contain PME. Culture filtrates were always negative for PME activity. No PME was detected after 7 or 14 days growth Bell (1951) have also found PME in healthy cucumber fruit. .pa

POLYGALACTRONASE :

SYNONYMES: Pectinase, Pectolases, Pectin Glycosidases, Pectin De polymerase etc.

PGs hydrolyzes the x - 1,4 glycosidic linkages of pectic substance.The action of these enzymes is assayed by measuring the increase in - CHO group, the decrease in molecular weight, the increase in mono & oligogalactronic acids, the decrease in optical activity, the decrease in viscocity, coagulability and jellying power etc.

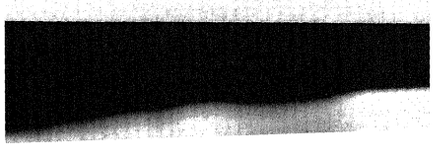
Demain & Phaff (1957) distinguished between the polygaloctronases preferring pectic acid or de-esterified parts of pectinic acid, as substrate and pectin methyl galactronase (PMG) . They further distinguished those polygalactronase as whether to cause cleavages in glycosidic linkages at random by an endotype of poly galactronase or polymethyl galactronase or an exotype of action in which only the end linkages are preferred.

Endo PG involvement has been found in vitro in culture media of P. aphanidermatum even within two days after its growth though varied with different media and days Indrasenan & Paily (1982) .They observed that conn's medium was the best for maximum enzyme production among five different media employed by them. Endo PG was noted by them on 4th day in different culture filtrates. They have also noticed the its presence in vivo and thus are of the view that it plays a decisive role in tissue maceration.

Winstead & Mc Comb (1961) found that in filtrates from cultures of P. aphanidermatum grown on media containing glucose as the carbon source markedly reduced the viscocity of Sodium poly pectate.

PECTIN TRANS ELIMENASE

The discovery of PTE was made by Albersheim et al (1960) PTE splits the pectic chain by a trans- eliminative mechanism. The enzyme breaks the glycosidic linkages of pectin at



C_4 accompanied by a simultaneous elimination of H^+ from C_5. Wood (1970) further classified these enzymes attacking preferably esterified or non esterified parts of the chains terminally (exo) or at random (endo). The viscocity reducing splits results in oligouronides

which terminates in a modified $C_{4,5}$ — unsaturated galactomethyl unit. The ability of the PTE to attack pectin varies with the degree of esterification of galacturonic acid chain, chain length and structural variation in the chain Albershiem & Killias (1962). Indrasenan & Paily (1982) have also recorded the production of these enzymes in vitro in varying concentrations on different media inoculated with P. aphanidermatum.

From the above description it is evident that the pectic enzymes plays an important role during pathogenicity within the host tissue.

With the above consideration the study in this section was planned under the following lines;

Sub-section (a) : In vivo studies of pectic enzymes

Under this sub section the presence and activity of pectolytic enzyme was studied both in healthy rhizomes and in the diseased rhizomes inoculated with Pythium aphanidermatum.

Sub section (b) : In vitro studies of Pectic enzymes under

various cultural condition

Under this sub section the capacity of pectolytic enzyme production by P. aphanidermatum was studied under varying cultural conditions ie., in different media, incubation period and the effect of pH.

Sub-section (c): In vitro studies of Pectic enzymes in presence of Growth Regulators & Fungicides:

Under this sub-section the effect of various plant growth regulators, and fungicides were studied on the pectolytic enzyme produced by P.aphanidermatum.

CHAPTER XI

REVIEW OF THE LITERATURE

The pectic enzymes plays an important role in tissue disintegration and thus involved in pathogenesis Truner & Bateman (1968) . According to the reports of Kertesz (1951) & Bateman & Millar (1966) pectic enzymes are not frequently found in the healthy tissue . Capellin (1966) found active secretion of PG by Rhizopus stolonifer which occurred during the active growth phase and ceased prior to the attainment of maximum growth. Tribe (1955) while working with the preparation of Bacterium ariodeae & Botrytis cinerea found that a metabolite, which presumably was an enzyme, kills the protoplasm. Pectic enzymes of Pseudomonas solanacearum play important role in pathogenesis in the break down of host tissues (Husain & Kelman, 1950).

Meceration of plant cells is an important phenomenon in the syndrome of disease caused by Pythium aphanidermatum (Brown, 1915). It is caused by dissolution of middle lamella composed largely of pectic substance resulting in the loss of coherence of plant tissues. De Bary (1886), while working on extracts of rotten tissue infected with Sclerotium enzyme may be involved in the pathological manifestation induced in plant tissue by pathogenic micro organism. Jones (1901) suggested soft rotting bacteria responsible for break down of infected tissue. Bateman (1963)worked on the pectolytic enzymes of Rhizoctonia solani in culture filrtrate and extract of infected tissues of beans. He concluded that the presence of higher activity of extra cellular enzymes in maceration and development of rots in bean seedling played significant role in the disease. Hancock et al (1964) found that onion rot organism viz Botrytis allia B. cinerea & B squamoea produced pectolytic enzymes in vitro and in vivo.

Brown (1915) while working on Botrytis cinera showed active enzyme preparation present in young mycelia as well as in broth media. Gupta (1956) observed that pectic enzymes of Pythium debaryanum were stimulated by adding NaCl in the culture media Waggoner & Dimond (1955) found that Fusarium oxysporum f.lycopersici produced polygalactronase and pectic methyl esterase on pectic medium. They recorded direct relation ship in growth and enzyme production, and concluded that PG & PME were supposed to be active during pathogenesis. Durajraj (1959) found high activity of pectic enzymes in vitro in contrast to in vivo, while working with the chillies rot organism. Colletotrichum capsici. Keen & Horton (1966) worked on Pyrenochaeta terrestri and reported that PG & PE were synthesized in liquid culture media and also in infected roots. Gupta & Gupta (1967) studied the wilt disease of paddy and pea crop caused by Gibberella fujikuroi and found active production of pectolytic enzymes on synthetic media, which were found to be adaptive in nature.

Cole (1956) studied the role of pectic enzymes in pathogenicity of Botrytis cinerea, Sclerotium fructigena & S. laxa. In vitro preparations, he observed that these fungi did not show much enzyme activity in media of apple decoction. In contrast the media which contain glucose and ammonium tartrate gave active enzyme preparation which could also be suppressed if apple extract was added to this medium. From this he concluded that there were some factors in apple juice which were antagonistic to enzyme activity Singh & Wood (1956) confirmed the findings of Cole (1956) by showing that Fusarium moniliformae secreted macerating enzymes when certain natural extracts, pectic substances or galacturonic acid were added to the liquid media.

The enzyme activity of these extracts was inhibited if apple extracts were originally added. Srivastava et al (1959) reported that pectolytic activity was higher in vivo preparation than that of any of the culture filtrates of Rhizopus stolonifer.

Sherwood (1966) while working on Rhizoctonia solani found correlation of pH to the production of pectic enzymes . Hancock et al (1966) found at pH6.2 to 7.1 & also upto pH 8.2 in case of Colletotrichum tirfolii. Wood (1960) reported that pectic enzymes may also be secreted in the absence of soluble or insoluble pectic substances.

Hancock (1966) ; Bateman (1966) and Mellano et al (1970) reported that pectolytic enzymes activity of root rot and damping off pathogen is involved in pathogenesis. Pectic enzymes of various parasitic fungi was studied by Menon (1934) Fernando (1937) worked on pectinase enzyme of some pathogen. Paff (1947) showed that Penicillium chrysogenum produced exo cellular pectic enzyme and that polygalactronase and pectin methyl esterases were adaptively produced. Sherwood (1964) was able to observe the production of PMTE & PG in case of Rhizoctonia solani and certain other fungi. Miller (1965) also observed PGTE production by Fusarium species which was isolated from alfa alfa roots. Ayers & Papaviazas (1965) worked on pathogenecity of Rhizoctonia species &found no correlation between their virulence and the enzymes of various isolates in vitro. Ayers & Papaviazas (1966) worked on different isolates of Fusarium oxysporium f. solani . Ashours (1954) made a comparative study of pectinase production in Botrytis cinera & Pythium debaryanum.

The subject has also been very extensively reviewed by Brown (1936, 1965) Wood (1960) Sadasivan & Subramaniam (1963-64) & Bateman & Millar (1966) and the enzymological approach of pectic enzymes have been very well described by Lineweaver and Tansen (1951).

Effect of fungicides and antibiotics on pectolytic enzyme activity of Rhizoctonia bataticola has been described by Goel & Mehrotra (1973 , 1974).

PART II

SECTION 'A'

SUB SECTION 'a'

CHAPTER XII

INTRODUCTION

INVIVO STUDIES OF PECTIC ENZYMES

Among the various cell wall degrading enzymes the pectolytic ones are considered to be of greater importance during the pathogenesis caused by fungal pathogens. Demethylation, hydrolytic or non-hydrolytic cleavage of chains of pectic substances as a result of the activity of various pectic enzymes often lead to irreversible changes in the cell wall composition of the host. The events are then followed by the development of disease syndrome. The secretion of these enzymes in the disease host tissues and in culture filtrate of many phytopathogenic fungi and their direct or indirect involvements in pathogenesis has already been established. In the present investigation the existance and activity of various pectic enzymes in healthy rhizomes and in Pythium aphanidermatum infected rhizomes have been studied.

CHAPTER XIII

EXPERIMENTAL

1. Preparation of extracts from healthy rhizomes

The healthy rhizomes were surface sterilized by dipping them in .1% Hg Cl_2 solution for 2 minutes after repeated washing with sterilized water. About 5 gms of the healthy tissue was taken and crushed in a mixture with equal volume of sterilized .5 N solution of NaCl for 5 minutes. The homogenate was filtered under suction, the extract thus obtained was centrifuged for 20 minutes at 4000 rpm. The supernatant liquid was then collected and dialysed. This extract was taken as enzyme preparation and was used for enzyme studies in healthy rhizomes.

2. Preparation of extract from infected rhizome:

Healthy rhizomes were first surface sterilised by dipping them in .1% Hg Cl2 solution for 2 minutes and after repeated washing (about 15) with sterilized distilled water were cut into 5mm slices with a sterilized blade, than asceptically transferred to the inoculum of the pathogen kept ready and prepared as under:

Inoculum was taken from the edge of 2-3 days old colony or the rhizome was placed in the sand oat meal inoculum as described in Chapter VI and kept in a 250 ml conical flask oand incubated at 26 C. These infected rhizomes were used for enzymatic study after 5 days and 10 days of incubation period. There replicates were taken simultaneously Uninoculated rhizomes were taken as control.

About 5 gms of the rotten tissue was taken, crushed in a mixer in equal volumes of sterilized 0.25 N solution of NaCl for about 5 minutes at room temperature. The homogenate was filtered under suction and the extract thus obtained was centrifuged for 20 minutes at 4000 rpm. The supernatant liquid was then collected and dialysed. This extract was taken as enzyme preparation and was used for enzyme study.It was stored and freezed at 4°C and enzymatic studies completed within 3 days. In each case a small rotten portion was placed in petridish containing PDA to resolute the pathogen so as to confirm infection and detect contamination if any.

The enzyme preparation obtained from healthy and diseased rhizome were subjected to various enzymatic assay for the activity of Protopectinase, Pectin Methyl Estrase, Poly Methyl Galactronase Trans - Eliminase and Pectin Methyl Trans-Eliminase as under:

1. Studies on Protopectinase:

For this fully turgid potato were emerged in the test solution in a breaker of 100 ml

Enzyme preparation was buffered at the required pH with Macllvain phosphate citrate buffer. The enzyme reaction mixture contained 1:4 mix of buffer and enzyme extract. Potato disc cut to a dia of 8 mm with a cork borer and .5mm in thickness by hand microtone were emerged in the above solution. To record activity time, time was observed for total loss of coherence in tissue disc. This was observed by lifting the tuber with dissecting needle, when the disc was not lifted the time for total loss in coherence was recorded at an average of 5 disc & was termed as "Reaction time." The macerating enzyme activity was determined as 1000/t where t = the time required for total loss of coherence.

Reaction time is recorded in Table VIII.

2. Studies on Pectin Methyl Esterase:

The activity was measured by the titration of the reaction mixture with .02 N NaOH with the help of a pH meter. A solution of 1.2% citrous pectin pH 5.5 with 1 N NaOH was taken as substrate. 30 ml of the substrate was taken and added with 4.5 ml of enzyme preparation and the pH of the reaction mixture recorded immediately as at the 0 hrs. After reaction time of 10, 20, 40 and 100 minutes the pH of the reaction mixture changes and it was noted. The enzyme substrate mixture was then titrated with .02 N NaOH to the pH recorded at 0 hrs and the amount of NaoH required was noted at each interval of incubation. The data recorded as micro equivalent of NaOH used at reaction time t/ml filtrate and reciprocated to 1000.

$$\text{REA at T} = \frac{.02 \text{ N NaOH}}{4.5} \times 1000$$

REA = Relative enzyme activity

T = Reaction time.

Studies are given in table IX.

3. Studies on Pectin Methyl GalactronaseThe enzyme activity was studied using Ostwald Viscometer. The substrate consist of :

1.2% Citrous pectin solution

The enzyme reaction mixture consists of :

— Citrous pectin solution — 3.5 ml

— Macllvain Buffer (5.5 pH) — 1.5 ml

— Distill water — 1.5 ml

— Enzyme preparation - 1.5 ml

Viscometer were fixed vertically in water bath at 25 C. The above component were poured in the bulb in the same sequence. Efflux time of enzyme reaction mix was determined at 5,15,30,60 & 90 minutes. This is refereed as reaction time .This mixture with autoclaved enzyme extract was run as control. Efflux time for 8ml of water was noted for each viscometer. The determination of the percent loss in viscocity & relative enzyme activity were calculated according to the following formula :

$$\% \text{ Loss in Viscocity} = \frac{Et_o - Et_t}{Et_o - Et_w} \times 100$$

Where Et_o = Efflux time at 0 hrs.

Et_t = Efflux time at time t.

Et_w = Efflux time for water

The results are observed in table X.

4. Studies on Poly Galactronase(PG):

In this case the substrate was 1.2% solution of Sodium Poly Pectate and reaction mix consist of —

1.2% Sodium poly Pectate = 3.5 ml

Macllvain Buffer = 1.5 ml

Distill water = 1.5 ml

Enzyme preparation = 1.5 ml

Reaction time at 5,15,30,60 & 90 minutes was studies for percent loss in viscocity and relative enzyme activity are recorded in table XI.

5. Studies on Poly Galactronase Trans Eliminase

Enzyme activity was analysed by viscometer and percent loss in viscocity was noticed. The substrate used was 1.2% sod. poly pectate solution at pH 8 .

The enzyme reaction mixture consist of —

1.2% Sod. poly pectate = 3.5 ml

Distill Water = 1.5 ml

Buffer pH 8 = 1.5 ml

Enzyme preparation = 1.5 ml

The enzyme determination of the percent loss of viscocity and relative enzyme activity were calculated according to the following formula :

$$\% \text{ Loss in Viscocity} = \frac{Et_o - Et_t}{Et_o - Et_w} \times 100$$

Where Et_o = Efflux time at 0 hrs.

Et_t = Efflux time at time t.

Et_w = Efflux time for water

PGTE activity is observed in the table XII.

6. Studies on Pectin Methyl Trans Eliminase:

Viscometerically enzyme was assayed using 1.2% pectin solution at pH8 Enzyme reaction mix consist of -

1.2 % pectic solution	= 3.5 ml
Distill water	= 1.5 ml
Buffer pH8	= 1.5 ml
Enzyme extract	= 1.5 ml

Calculation of the efflux time is same as above. Observations recorded in Table XIII.

CHAPTER XIV

RESULTS AND OBSERVATIONS

Observations were made for the production and activity of Protopectinase, Pectin Methyl Estrase, Polygalactronase, Pectin Methyl Galactronase, Poly Galactronase Transeliminase and Pectin Methyl Trans-Eliminase. The results obtained are tabulated in the tables VIII - XIII & Fig. VI-XIII.

The macerating activity of the enzyme Protopectinase was determined on potato disc when dipped in filtrates obtained from healthy and diseased rhizome infected by P. aphanidermatum. The healthy rhizome extract was unable to produce loss of coherence in potato discs upto 5 hrs the time for which observations were made. However, filtrates obtained from the infected rhizomes Protopectinase activity was noticed. The activity was higher after 5 days of incubation as compared to that of 10 days (Table VIII).

PME activity was also quite insignificant in fresh rhizomes. However infected rhizomes did produce some enzyme activity. Theactivity was higher after 5 days of incubation as compared to 10 days of incubation. Similar were the results of Winstead & Mc Combs (1961), Bell (1951). Increasing the reaction time the enzyme substrate mixture after 10, 20, 40 & 100 minutes did not produce any change in the percentage loss in viscocity (Table - IX).

Enzyme activity for PG, showed a higher activity after 5 days of incubation . The fresh ginger rhizome also gave PG activity which was slightly lower than that obtained after 10 days of incubation (Table XI, Fig. VI & VII).

Enzyme activity in case of PMG, PMTE & PGTE was found to be increased after 10 days of incubation as compared to that of 5 days . Here again the fresh rhizomes did show the enzyme activity but was not very significant when compared with the diseased rhizome. (Table X, XII & XIII, Fig. VIII - XIII).

From the above results it appears that glycosidases are more active than transeliminases during pathogenesis. Regarding the affect of incubation period it was noticed that Protopectinase, P.M.E. & P.G. showed maximum activity on 5 days infected rhizome while PMG, PGTE & PMTE showed maximum activity on 10 days old infected rhizomes.

TABLE VIII

PROTOPECTINASE ACTIVITY ON GINGER RHIZOME

SNO	DAYS AFTER INCUBATION	REACTION TIME	MACERATING ENZYME ACTIVITY
1.	0	not macerated upto 5 hrs.	----
2.	5	16.3 min	6.13
3.	10	223 min	4.48

TABLE IX

PECTIN METHYL ESTERASE ACTIVITY ON GINGER RHIZOME

SNO	DAYS OF INCUBATION	NAOH REQUIRED AFTER MINUTES			
		10	20	40	100
1.	0	3.0ml	3.0ml	3.0ml	3.0ml
2.	5	9.0ml	9.0ml	9.0ml	9.0ml
3.	10	3.7ml	3.7ml	3.7ml	3.7ml

TABLE X

PECTIN METHYL GALACTRONASE ACTIVITY IN GINGER RHIZOME INOCULATED WITH P.aphanidermatum

SNO	DAYS OF INCUBATION	% LOSS IN VISCOCITY AFTER MINUTES					REA
		5	15	30	60	90	
1.	0	4.5	4.5	5.8	5.8	5.8	---
2.	5	5	6.5	7	10	10.5	---
3.	10	6.5	12.3	18.7	30	40.5	22.22

REA AT 25% LOSS IN VISCOCITY

TABLE XI

POLY GALACTRONASE ACTIVITY IN GINGER RHIZOMES INOCULATED WITH P.aphanidermatum

SNO	DAYS OF INCUBATION	% LOSS IN VISCOCITY AFTER MINUTES					REA
		5	15	30	60	90	
1.	0	5	7.2	10	11.8	12.5	----
2.	5	15	20.1	25.4	35.3	40	35.71
3.	10	7	9	12.5	15	18	----

REA AT 25% LOSS IN VISCOCITY

TABLE XII

PGTE IN GINGER RHIZOME INOCULATED WITH P.aphanidermatum

SNO	DAYS OF INCUBATION	% LOSS IN VISCOCITY AFTER MINUTES					REA
		5	15	30	60	90	
1.	0	3.5	5.0	6.0	6.0	6.5	-
2.	5	4.4	6.0	6.8	7.5	10.0	-
3.	10	3.5	10.4	12.0	18.0	25.3	11.11

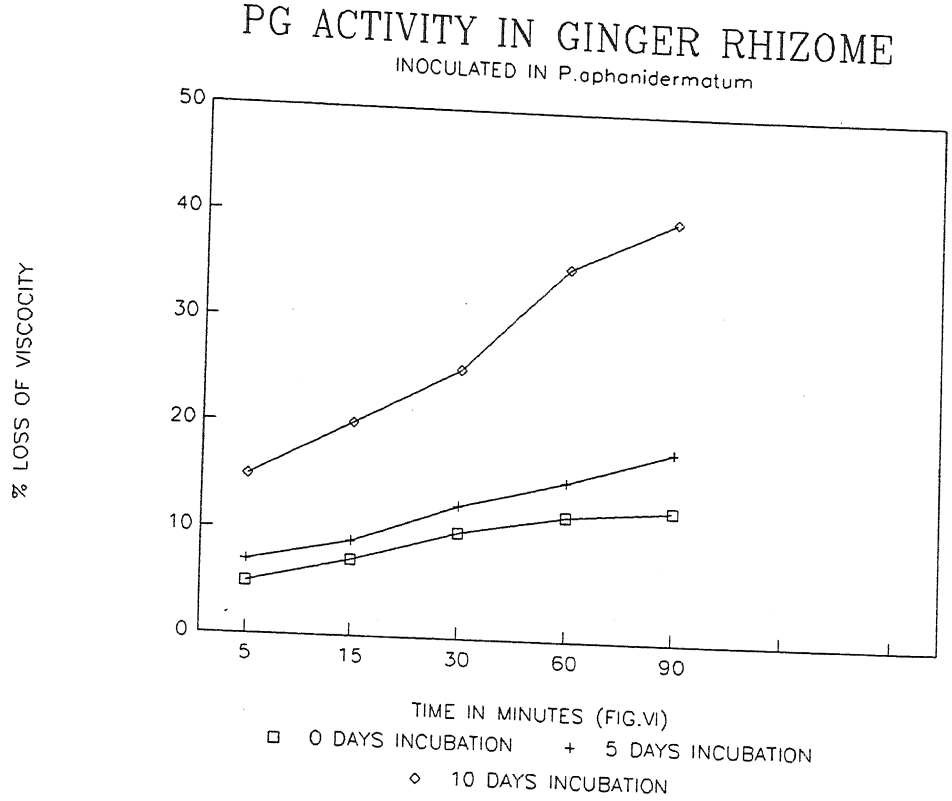
REA AT 25% LOSS IN VISCOCITY

TABLE XIII

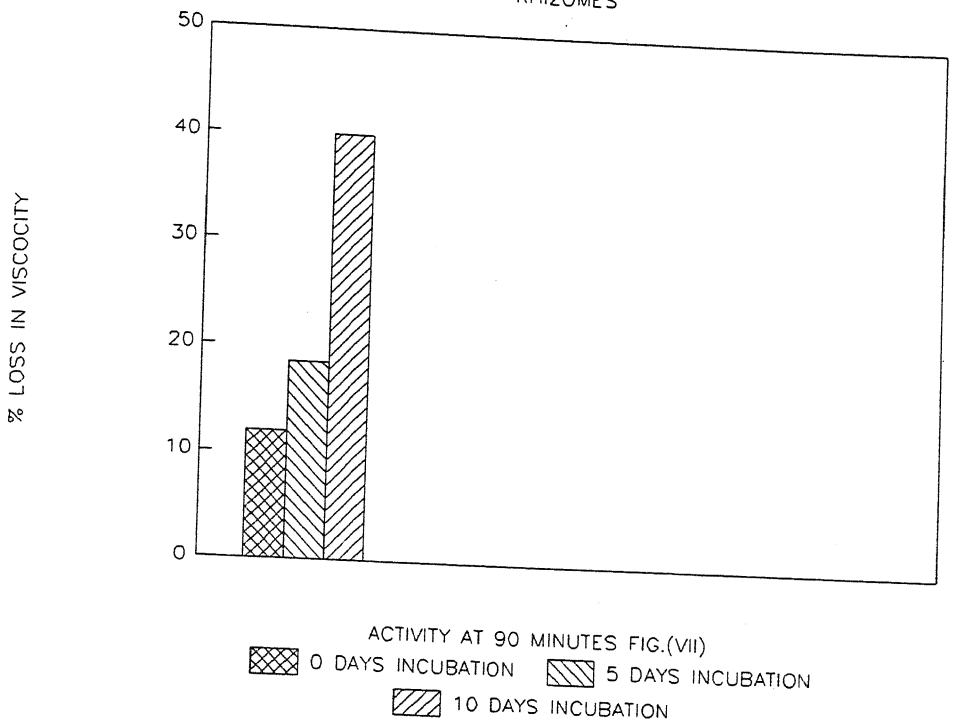
PMTE ACTIVITY IN GINGER RHIZOME INOCULATED WITH P.aphanidermatum

SNO	DAYS OF INCUBATION	% LOSS IN VISCOCITY AFTER MINUTES					REA
		5	15	30	60	90	
1.	0	4.0	5.7	6.0	6.5	7.0	--
2.	5	5.5	6.0	6.2	7.0	7.5	--
3.	10	7.0	13.2	15.2	20.1	25.0	40.0

REA AT 25% LOSS IN VISCOCITY

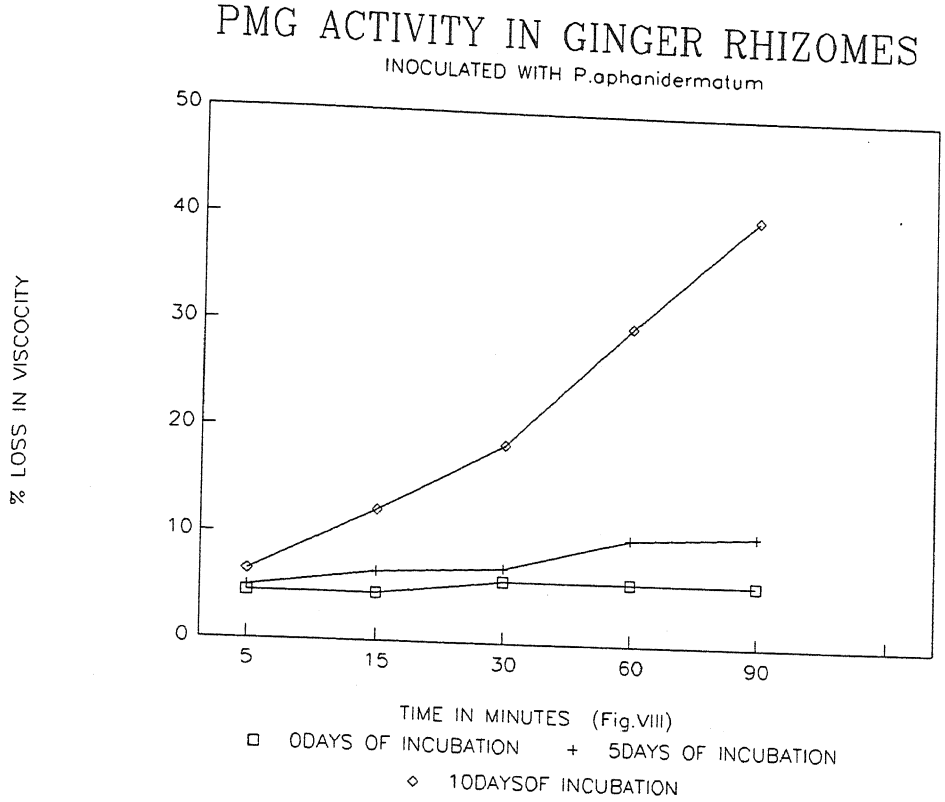

PG ACTIVITY IN GINGER RHIZOME
INOCULATED IN P.aphanidermatum
% LOSS OF VISCOCITY
50
40
30
20
10
0
5
15
30
60
90
TIME IN MINUTES (FIG.VI)
□ 0 DAYS INCUBATION
+ 5 DAYS INCUBATION
◇ 10 DAYS INCUBATION

PG ACTIVITY IN HEALTHY & IN INFECTED RHIZOMES

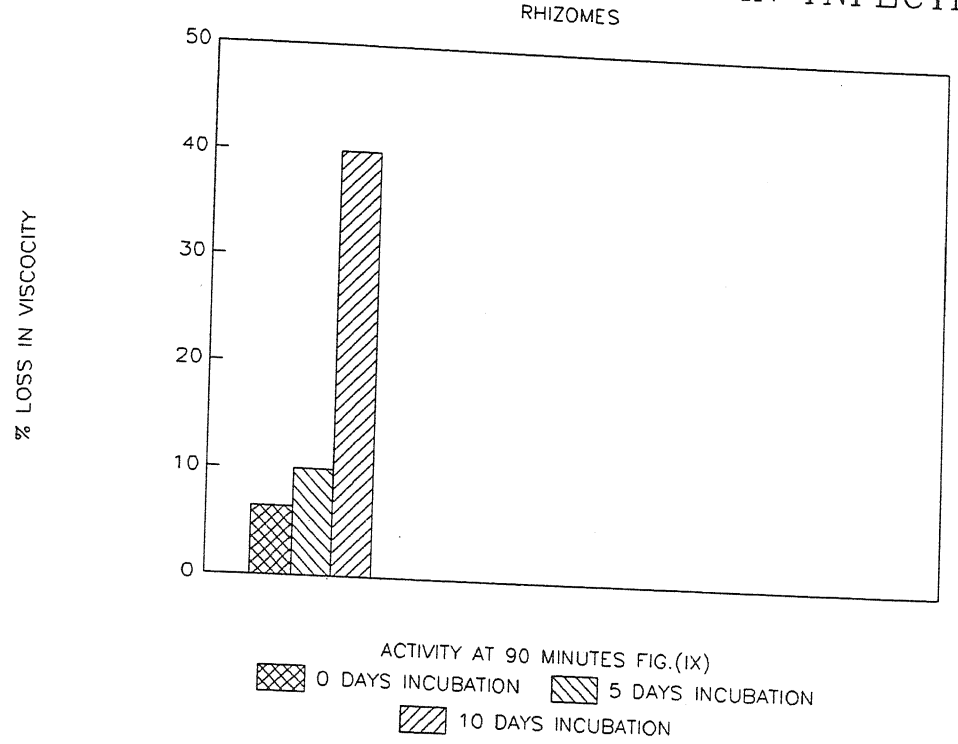


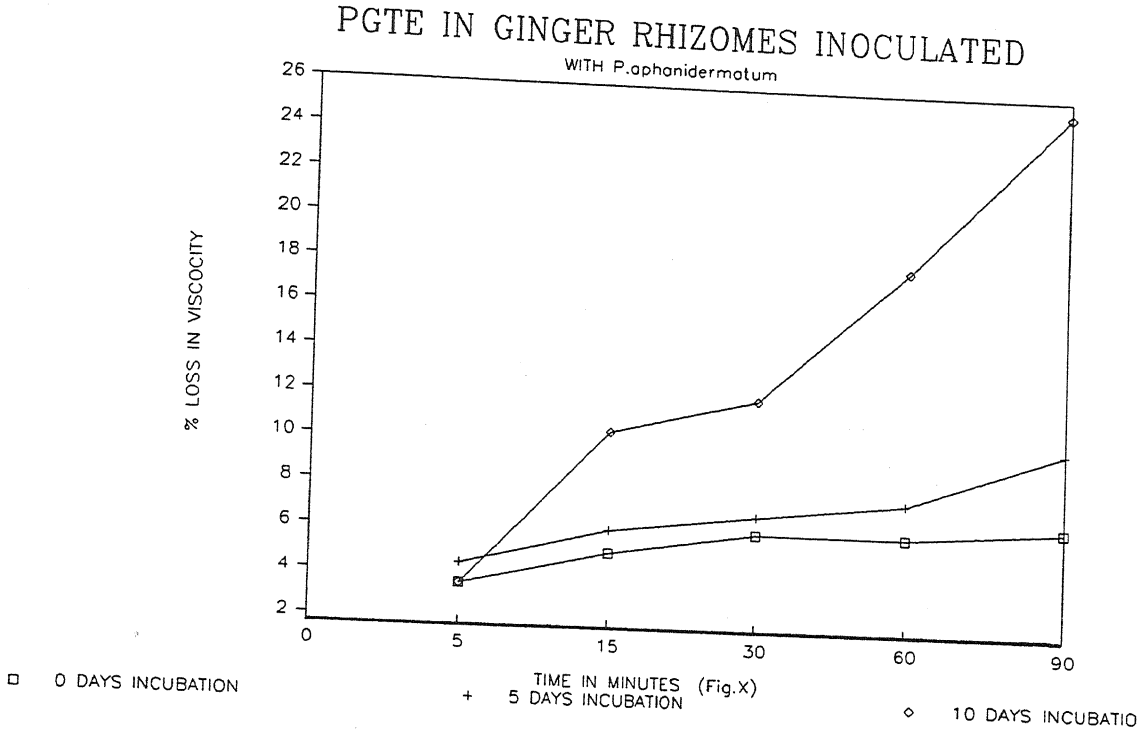
ACTIVITY AT 90 MINUTES FIG.(VII)

0 DAYS INCUBATION 5 DAYS INCUBATION 10 DAYS INCUBATION

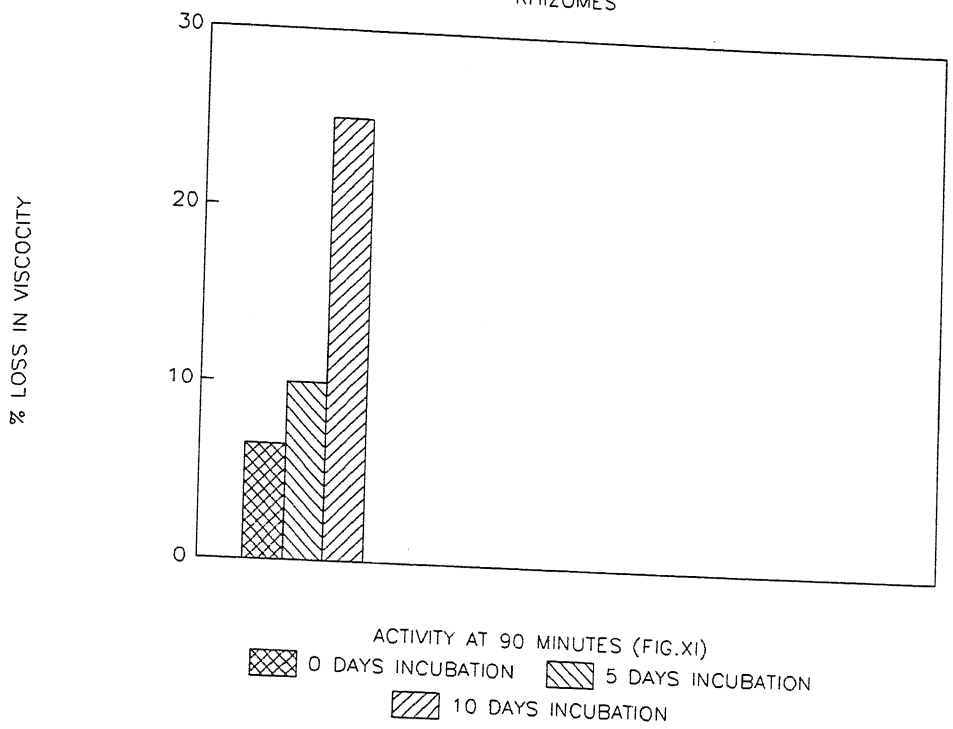

PMG ACTIVITY IN GINGER RHIZOMES
INOCULATED WITH P.aphanidermatum
% LOSS IN VISCOCITY
50
40
30
20
10
0
5
15
30
60
90
TIME IN MINUTES (Fig.VIII)
□ 0DAYS OF INCUBATION + 5DAYS OF INCUBATION
◇ 10DAYSOF INCUBATION

PMG ACTIVITY IN HEALTHY & IN INFECTED



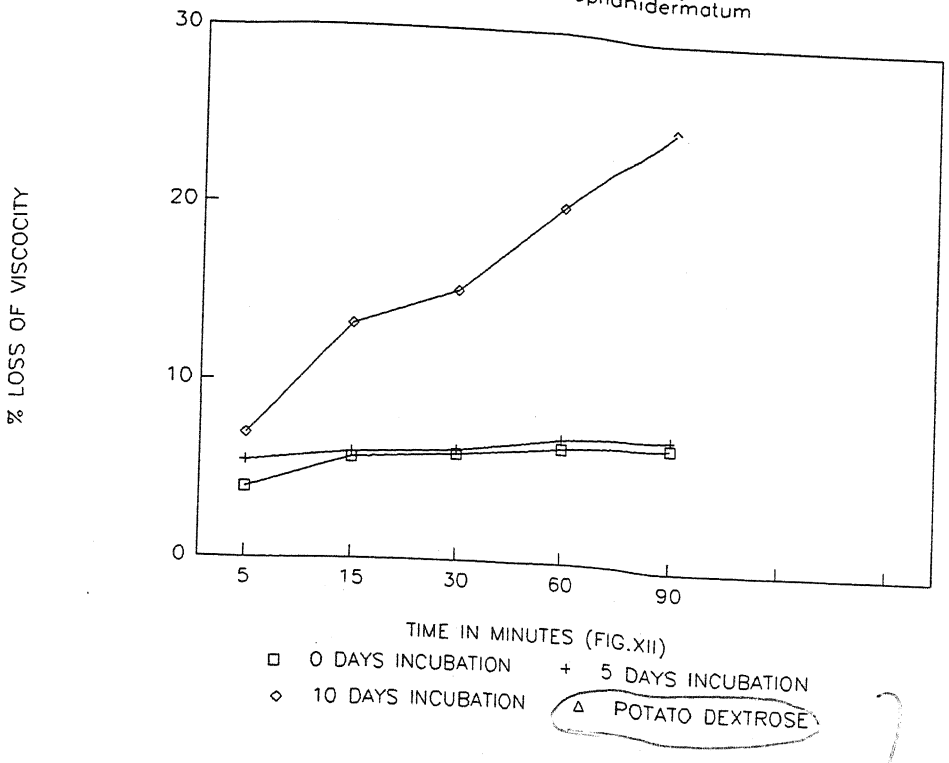

PGTE IN GINGER RHIZOMES INOCULATED
WITH P.aphanidermatum
% LOSS IN VISCOCITY
26
24
22
20
18
16
14
12
10
8
6
4
2
0
5
15
30
60
90
TIME IN MINUTES (Fig.X)
□ 0 DAYS INCUBATION
+ 5 DAYS INCUBATION
◇ 10 DAYS INCUBATIO

PGTE ACTIVITY IN HEALTHY & IN INFECTED RHIZOMES



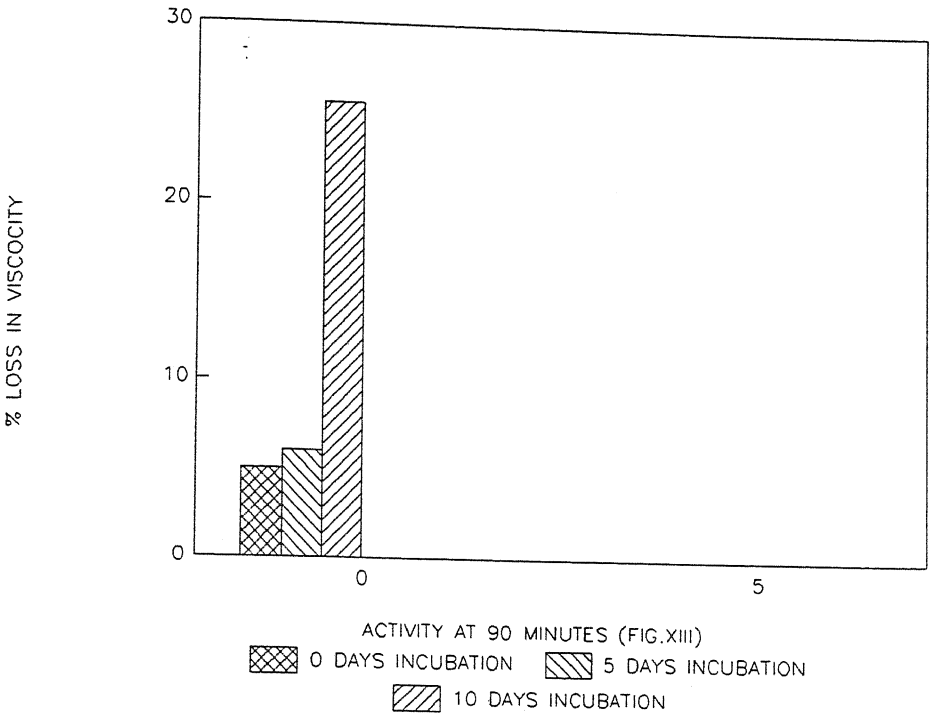
PMTE ACTIVITY IN GINGER RHIZOME

INOCULATED IN P.aphanidermatum



PMTE IN GINGER RHIZOME INOCULATED WIT

P.aphanidermatum



CHAPTER XV

DISCUSSION & CONCLUSION

The involvement of Pectic enzymes during pathogenesis of ginger rhizomes, the production & activity of various pectic enzymes in healthy and diseased rhizomes were investigated.

Pectic Enzymes in healthy rhizomes:

The extracts of healthy rhizomes showed the presence of 4 pectic. enzymes namely PG, PMG, PGTE & PMTE and slight activity of PME . Though the enzyme activity was not very significant when compared with the diseased rhizome. However PG was more active than the others. The presence of Glycosidases and lipases in certain healthy tissue have also been reported by Kertez (1951). PG activity was observed by Rai (1971) in healthy Pappaya fruits while Ali (1970) noticed only PME in healthy chilli fruits., Thus, the presence of various pectic enzymes as observed by the author is in line with the investigations of the above workers. Pectic substances undergo changes during the development of ginger rhizome and thus pectic enzyme activity observed by the author may be associated with the above changes during the development and ripening i.e., the so called Protopectin - pectin transformation.

Pectic Enzyme in Infected Rhizome:

The enzyme activity of all the six enzymesstudied showed much greater activity in diseased tissue as compared to the healthy one . From these results it can be concluded that the development of soft rot of ginger was associated with the increased activity of pectolytic enzyme. The presence of higher degree of pectolytic enzyme in diseased tissue has been recorded by many workers. Ali (1970),Rai (1971),Kertesz(1951), Indrasenan & Paily (1982), Winstead & McCombs(1961) , Bateman & Millar (1966), Husain & Kelman (1957). The observations of these workers lead to similar conclusion that pectic enzyme play important role in pathogenesis.

Comparatively higher activity of glycosidases than transeliminases suggest that the cleavage of both methylated and non methylated pectic chains are more activated by hydrolytic enzymes in infected rhizomes.

PART II

SECTION A

SUB SECTION 'b'

CHAPTER XVI

INTRODUCTION

INVITRO STUDIES OF PECTIC ENZYME

UNDER VARIOUS CULTURAL CONDITIONS

Certain parasites such as Botrytis cinerea & Bacterium ariodae produce pectolytic enzymes on a great variety of media, natural or synthetic and over a wide range of conditions. In the case of Pythium de Baryanum, the earlier workers Chona (1932), Menon (1934) & Fernando (1937) who studied the physiology of this fungus prepared active extract from parasitized potato tissue. Later, Ashour (1949, 1954) developed a synthetic medium, based upon the crude chemical composition of potato tubers, which was suitable for enzyme production. The notable feature of this medium being that any change upwards or downwards in concentration of its constituents caused a pronounced fall off in secretion of enzyme.

Damel (1952), while working with Ashours synthetic medium found that active secretion of enzyme was not obtained unless the glucose constituents was autoclaved with phosphate (K_3PO_4) & K_2HPO_4). Autoclaving with acid phosphate was in effective even when the medium was subsequently adjusted to a pH favourable for the growth of the fungus.Damel(1952)noted that when the medium was prepared in such a way that the carbohydrates (glucose & Fructose) was autoclaved with tribasic or dibasic phosphate (with or without other constituents) it developed a yellow to brown colour . Hence,colouration was a sign that the medium was suitable for vigorous secretion of enzyme.

Gupta S.C. (1956),found that active culture filtrates were obtained only when the carbon and nitrogen constituents of synthetic media were carefully balanced. Enzyme secretion was increased when Sodium Chloride was added and when the medium containing glucose as the carbon source was autoclaved at relatively high pH values. Less active filtrates were obtained when glucose was replaced by a high methoxyl pectin.

The capacity of Botrytis alli, B. cinera.,B. squamosa to produce pectolytic enzymesin Potato Dextrose Broth (PDB) in detached onion leaf sections, and in leaves of intact onion plants was examined by Hancock, J.G., R.L. Millar, and J.W. Lorbeen (1964). All the 3 species produced Pectin Methyl Esterase under these conditions. B. alli produced enzymes of the PG type,only in trace amounts when cultured in PDB, but production ofbothexo and endogalactronase in detached leaves and leaves of intact onions. B. cinerea produced a very active endo-PG under all 3 cultural conditions. Exo-PG was produced on detached onion leaves,. The activity of exo-PG was much less in extracted materials from leaves.

Production of pectic enzymes by pathogenic fungi in culture medium has been demonstrated by many workers as listed above. During these studies it has been observed that the secretion of enzymes by fungi in culture are largely influenced by various components present in the media. The various components of the medium variously influence the production of enzymes. The other factors not related to the components are the pH of the medium, the incubation period, and the temperature of incubation. Since, the disease develops at a lower temperature i.e., 26 ± 2°C was thought to observe the development of various pectic enzymes on the same temperature. The pH of the soil changes from place to place i.e., varies according to the soil as such. It was desirable to find out the enzymes, therefore the studies were aimed for various culture medium and incubation period. The incubation period was the one which was favourable to the disease development. The effect of different pH in the finally selected media was carried out in the present study.

CHAPTER XVII

EXPERIMENTAL

Still cultures of the pathogen were grown in broth media in 100 ml conical flask containing 35 ml of the medium and incubated with the pathogen. The flask were incubated at 26° C in triplicates. After 5 days the culture filtrate and mycelial mat was collected separately by filtration under suction on previously weighted filter paper . These cell free filtrates were than centrifuged at 4000 rpm for 20 minutes,and the supernatant liquid was taken for enzyme preparation and if required stored at 4° C for short period. The mycelial mat collected were given a thorough washing with distill water . These filter papers with mycelial mat were then placed in oven at 70° C and dried to constant weight. The dry weight of the mycelia was calculated. These studies were carried out to determine the influence of various cultural conditions upon the capacity of enzyme production of the pathogen as well as upon the growth of the pathogen.

Different culture media taken for the study are -

a. Potato Dextrose Agar b. Peptone Dextrose Agar

c. Glucose Asparagine

d. Czapecks Dox.

Methods for Enzyme Assay:

Enzyme extracts thus obtained from the various sources were assayed for the estimation of Protopectinase, Pectin Methyl estrase, Pectin Methyl Galactronase, Poly Galactronase, Poly Galactronase Trans Eliminase, Pectin methyl Trans Eliminase. To measure the enzyme activity the methods used were same as in the previous section.

Effect of pH:

The pathogens were grown in Glucose Asparaginemedium with pH range from 2.5 - 12. The pH of the medium was adjusted with .1N NaOH and .1 N HC1 and buffered with Macllvain Buffer of the

desired pH for values upto pH 8 and with standard Phospahate buffer .

For higher values,incubation was done upto 5 days at 26 ± 2° C. The extraction of enzymeswith the method of enzyme assay was the same as described previously.

CHAPTER XVIII

RESULTS AND OBSERVATIONS

Effect of different culture media on the production of various pectolytic enzymes by P. aphinadermatum

The effect of culture media on Protopectinase, PG, PMG, PGTE & PMTE were observed in different culture media. The data obtained are recorded in (Table XIV - XXII Fig. XIV - XXXVII) for 5 & 10 days of incubation period.

The above result show that the amount of activity of these enzymes were variable. Protopectinase activity was found to be best on 5th day of incubation period and reduced as the incubation period was increased to 10 days maximum activity was recorded on Potato Dextrose followed by Czapecks Dox, Peptone Dextrose and Glucose Asparagin respectively.(Table XIV)

No PME activity in vitro was observed on all the 4 medium tested either after 5 days or after 10 days of incubation.

The data recorded for PG activity, showed that maximum activity was observed on Glucose Asparagine medium,. Peptone Dextrose was next followed by Czapecks Dox and the least activity was recorded in Potato Dextrose Medium. The activity was less pronounced after 5 days of incubation, as compared to the 10th day except in Glucose Asparagine where the activity was more in 10th day culture filtrate as compared to the 5th day.(Table XVI & Fig XXII - XXV).

The production of another Glycosidases (PMG) was however found to be favoured most in Czapecks Dox after 5 days of incubation and in Glucose Asparagine after 10 days of incubation. Here both these mediums gave good results as compared to Peptone Dextrose and Potato Dextrose. (TableXV & Fig. XIV - XVII).

With regards to the production of transeliminases it was observed that Glucose Asparagine produced the maximum amount of PGTE followed by Peptone Dextrose. Here the activity was higher in 10 days of incubation as compared to the 5th day in all the 4 mediums studied. Table XVII.& Fig XVIII - XXI.

While studying the response of various medium for PMTE production it was observed that Glucose Asparagine produced the maximum amount of PMTE followed by Czapecks Dox and Peptone Dextrose.

The enzyme activity was higher on 10th day of incubation as compared to 5th days of incubation periods. Table XVIII & Fig. XXVI - XXIX.

It is clear from the above data that P. aphinadermatum produced variable pectic enzymes under different incubation period and with different culture media. Glucose Asparagine medium was proved to be the best for PG, PMG, PGTE & PMTE. Incubation period of 5 days however appears to be optimum for PG & PMG activity while 10 days was found to be optimum for PGTE & PMTE activity.

Effect of pH on the production of pectic enzyme by P. aphanidermatum upon culture filrtates:

The data recorded on the various culture medium studied above show that Protopectinase activity was not very significant and -

- PME activity was not at all observed in any of the medium,. Thus the studies on two enzymes were not undertaken while studying the pH. The results showing the affect of pH of the culture media on the production of PG, PMG, PGTE & PMTE are given in table XIX, XX, XXI, XXII & Fig.XXX-XXXVII.

It is evident from the data recorded in Tables XIX & XX both the glycosidases PG & PMG were actively produced at pH range 3-7. The maximum activity was recorded at pH 4 & 5 respectively. Comparatively PMG was more sensitive than PG to alkaline pH range as no PMG activity was observed beyond pH8 whereas PG activity observed at pH9.

The production of Transeliminases was however favoured in the alkaline range of the medium. The maximum activity of both PGTE & PMTE was observed at pH 8-9 in both the cases. Table XXI, XXII Fig. XXXIV-XXXVII. -

-The pH range below 8 and above 9 appear to be very much unfavourable for those enzymes. PGTE was more active tham PMTE and relatively PMTE was more sensitive for pH changes.

TABLE XIV

EFFECT OF DIFFERENT CULTURE MEDIA ON PROTO PECTINASE ACTIVITY

MEDIUM	REACTION TIME (AFTER INCUBATION)		MACERATING ACTIVITY 1000/T(AFTER INCUBATION)	
	5DAYS	10 DAYS	5DAYS	10 DAYS
POTATO DEXTROSE	93min	175min	10.75	5.71
PEPTONE DEXTROSE	180min	not macerated upto 5hrs	5.55	----
GLUCOSE ASPARAGIN	200min	not macerated upto 5hrs.	5.00	----
CZAPECKS DOX	109min	205minutes	9.17	4.87

TABLE XV

EFFECT OF DIFFERENT CULTURE MEDIA ON PECTIN METHYL GALACTRONASE PRODUCTION BY P.aphanidermatum

MEDIA	PECTIN METHYL GALACTRONASE ACTIVITY AFTER 5 & 10 OF INCUBATION											
	5DAYS % LOSS OF VISCOCITY AFTER MIN					REA	10DAYS % LOSS OF VISCOCITY AFTER MIN					REA
	5	15	30	60	90		5	15	30	60	90	
POTATO DEXTROSE	6.0	7.0	7.5	9.0	9.5	-	3.0	5.2	6.3	6.3	7.0	-
CZAPECKS DOX	7.0	9.0	15.8	18.3	20.7	-	5.0	8.0	12.2	15.5	15.5	-
PEPTOSE DEXTROSE	5.8	5.8	6.5	7.3	8.0	-	6.0	6.5	6.5	9.0	11.0	-
GLUCOSE ASPARAGIN	4.0	6.9	10.1	11.3	15.0	-	7.2	10.5	13.7	18.1	22.5	-

REA AT 25% loss in viscocity.

TABLE XVI

EFFECT OF DIFFERENT CULTURE MEDIA ON POLY GALACTRONASE PRODUCTION BY P.aphanidermatum

MEDIA	POLY GALACTRONASE ACTIVITY AFTER 5 & 10 OF INCUBATION											
	5 DAYS % LOSS OF VISCOCITY AFTER MINUTES					REA	10 DAYS % LOSS OF VISCOCITY AFTER MINUTES					REA
	5	15	30	60	90		5	15	30	60	90	
POTATO DEXTROSE	4.5	6.1	8.8	14.2	16.5	–	3.2	5.0	5.0	8.3	9.1	–
CZAPECKS DOX	7.0	8.5	10	21	23.2	–	1.8	4.0	5.2	5.5	5.5	–
PEPTOSE DEXTROSE	8.3	12.0	19.4	28.1	35.3	20.20	10.0	15.1	18.5	20.0	24.5	–
GLUCOSE ASPARAGIN	16.0	18.0	28.1	30.0	37.2	39.20	15.0	28.0	34.0	36.0	46.0	15.3

TABLE XVII

EFFECT OF DIFFERENT CULTURE MEDIA ON POLY GALACTRONASE TRANSELIMINASE PRODUCTION BY P.aphanidermatum

S.NO	PMTE ACTIVITY AFTER 5DAYS & 10 DAYS OF INOCULATION											
	5DAYS						10DAYS					
MEDIA	% LOSS IN VISCOCITY AFTER MIN					REA	%LOSS IN VISCOCITY AFTER MIN					REA
	5	15	30	60	90		5	15	30	60	90	
1.POTATO DEXTROSE	4.0	4.2	5.0	6.8	7.1	--	3.5	4.0	5.1	7.5	9.0	--
2. CZAPECKS DOX	3.1	4.5	5.2	6.0	6.5	--	8.0	9.5	10.2	11.1	11.8	--
3.PEPTOSE DEXTROSE	2.0	2.4	5.4	6.8	8.0	--	6.5	8.1	12.5	14.0	14.2	--
4.GLUCOSE ASPAR - AGIN	9.5	14.0	14.5	15.2	16.0	--	12.5	13.8	15.0	19.8	21.1	--

REA AT 25% LOSS IN VISCOCITY

TABLE XVIII

EFFECT OF DIFFERENT CULTURE MEDIA ON PECTIN METHYL TRANSELIMINASE

PRODUCTION BY P.aphanidermatum

PMTE ACTIVITY AFTER 5DAYS & 10 DAYS OF INOCULATION

SN	MEDIA	5DAYS %LOSS IN VISCOCITY AFTER MIN 5	15	30	60	90	REA	10DAYS %LOSS IN VISCOCITY AFTER MIN 5	15	30	60	90	REA
1.	POTATO DEXTROSE	3.0	4.8	5.8	5.8	6.2	---	3.0	3.2	3.5	4.0	4.1	---
2.	CZAPECKS DOX	4.5	5.6	6.0	8.5	9.1	---	8.1	15.8	17.2	18.8	21.5	---
3.	PEPTOSE DEXTROSE	1.5	1.7	1.7	1.8	2.2	---	3.5	4.2	5.8	6.1	7.2	---
4.	GLUCOSE ASPARAGIN	4.0	6.1	8.5	9.1	14.8	---	9.3	16.0	16.8	18.5	28.2	44.44

REA AT 25% LOSS IN VISCOCITY

TABLE XIX

EFFECT OF pH ON POLYSALACTINASE PRODUCTION

INITIAL pH	% Loss In Viscocity after minutes				
	5	15	30	60	90
2.5	---	---	---	--	---
3	15.0	20.3	25.2	27.3	30.4
4	18.0	22.4	28.1	30.8	35.6
5	20.0	25.1	30.3	35.6	40.2
6	16.0	18.0	28.1	30.0	37.2
7	16.5	21.2	26.8	29.2	38.4
8	12.4	20.1	22.0	26.1	28.5
9	2.3	4.0	5.2	7.6	8.0
10	---	---	---	---	---
11	---	---	---	---	---
12	---	---	---	---	---

TABLE XX

EFFECT OF pH ON PECTIN METHYL GALACTRONASE PRODUCTION

Initial pH	% Loss In Viscocity after minutes				
	5	15	30	60	90
2.5	---	---	---	--	---
3	8.2	9.0	10.3	15.1	20.5
4	12.0	17.2	17.8	25.3	30.2
5	15.2	20.6	26.3	29.4	35.5
6	15.8	21.5	23.2	24.0	25.2
7	14.0	15.2	18.1	18.2	20.5
8	5.1	6.2	6.2	8.5	9.2
9	---	---	---	---	---
10	---	---	---	---	---
11	---	---	---	---	---
12	---	---	---	---	---

TABLE XXI

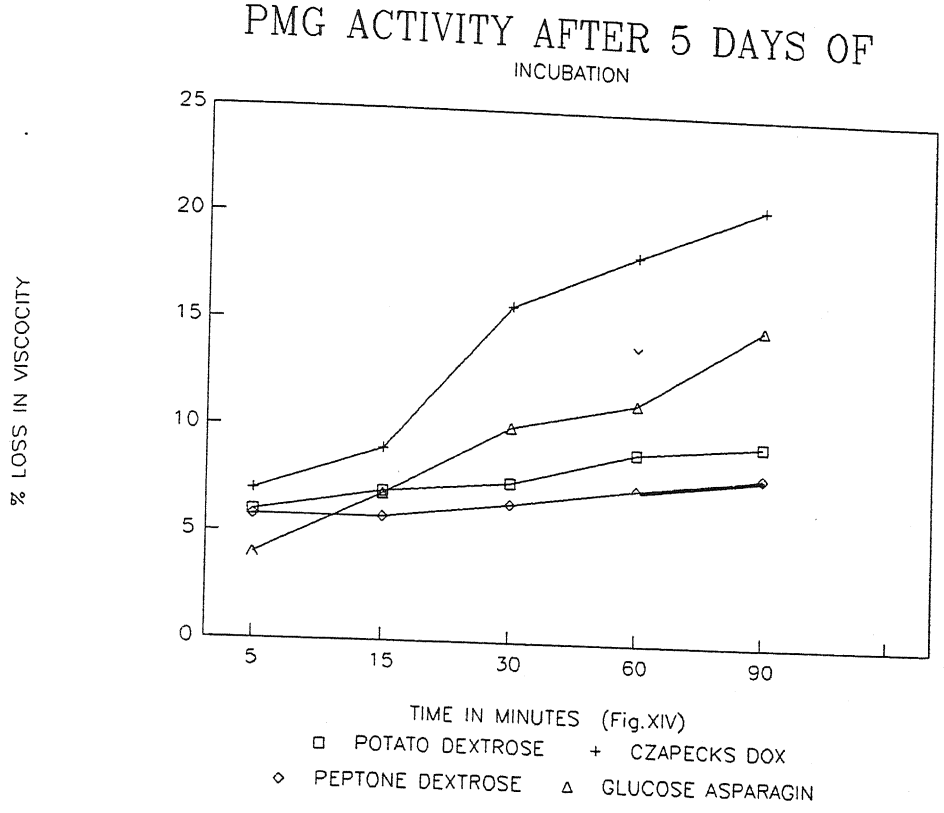
EFFECT OF pH ON POLYGALACTRONASE TRANSELIMINASE PRODUCTION

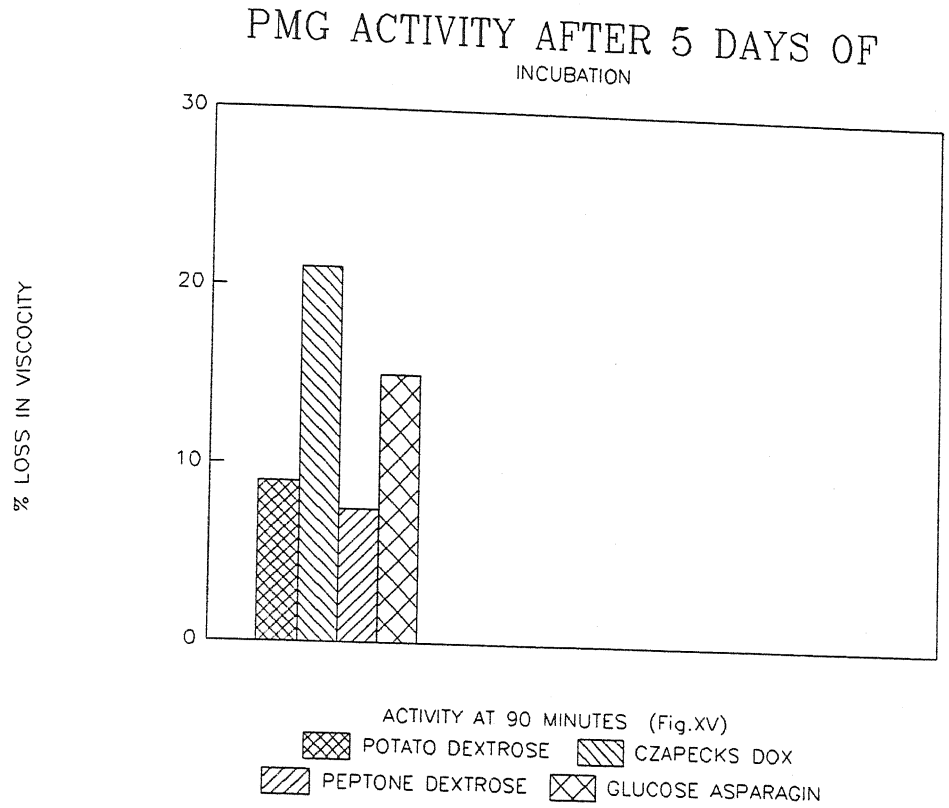
INITIAL pH	% Loss In Viscocity After Minutes				
	5	15	30	60	90
2.5	---	---	---	--	---
3	---	---	---	---	---
4	3.0	3.5	5.2	6.1	6.1
5	4.0	6.4	8.7	9.2	12.5
6	3.8	6.0	6.1	10.5	13.2
7	6.1	8.2	10.4	13.2	15.8
8	9.2	18.1	20.4	25.3	34.8
9	8.7	20.1	23.4	23.4	30.5
10	4.2	8.5	11.5	17.8	20.1
11	2.1	2.5	3.2	3.2	3.5
12	---	---	---	---	---

TABLE XXII

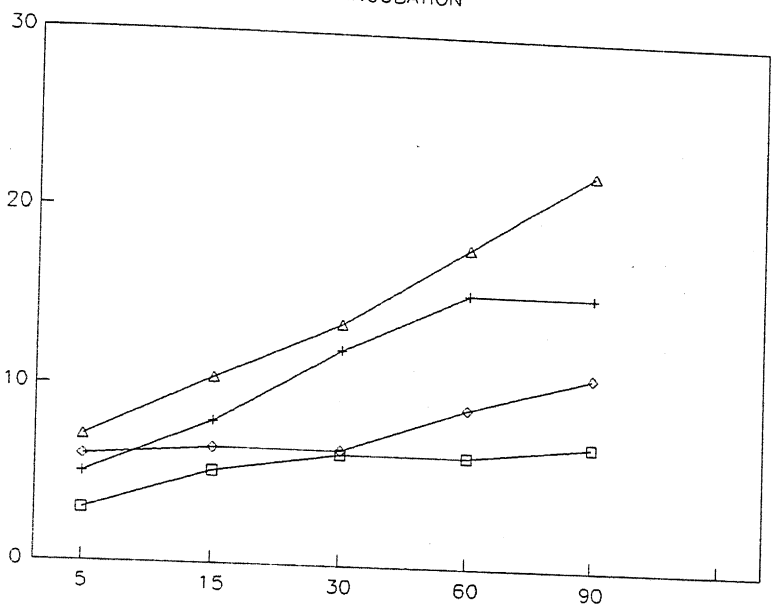
EFFECT OF pH ON PECTIN METHYL TRANSELEMINASE

INITIAL	% Loss In Viscocity after minutes				
ph	5	15	30	60	90
2.5	---	---	---	--	---
3	1.5	2.2	2.2	4.1	5.0
4	3.1	4.7	6.4	7.8	10.5
5	4.1	6.2	8.4	10.1	14.0
6	6.5	8.2	8.2	10.7	15.2
7	5.1	5.7	6.5	8.4	10.2
8	8.4	10.1	12.5	18.4	25.4
9	6.5	8.1	8.1	12.2	15.4
10	3.4	3.4	4.2	4.2	4.2
11	---	---	---	---	---
12	---	---	---	---	---


PMG ACTIVITY AFTER 5 DAYS OF
INCUBATION
% LOSS IN VISCOCITY
25
20
15
10
5
0
5
15
30
60
90
TIME IN MINUTES (Fig.XIV)
□ POTATO DEXTROSE
+ CZAPECKS DOX
◇ PEPTONE DEXTROSE
△ GLUCOSE ASPARAGIN


PMG ACTIVITY AFTER 5 DAYS OF
INCUBATION
% LOSS IN VISCOCITY
30
20
10
0
ACTIVITY AT 90 MINUTES (Fig.XV)
POTATO DEXTROSE
CZAPECKS DOX
PEPTONE DEXTROSE
GLUCOSE ASPARAGIN

PMG ACTIVITY AFTER 10 DAYS OF INCUBATION



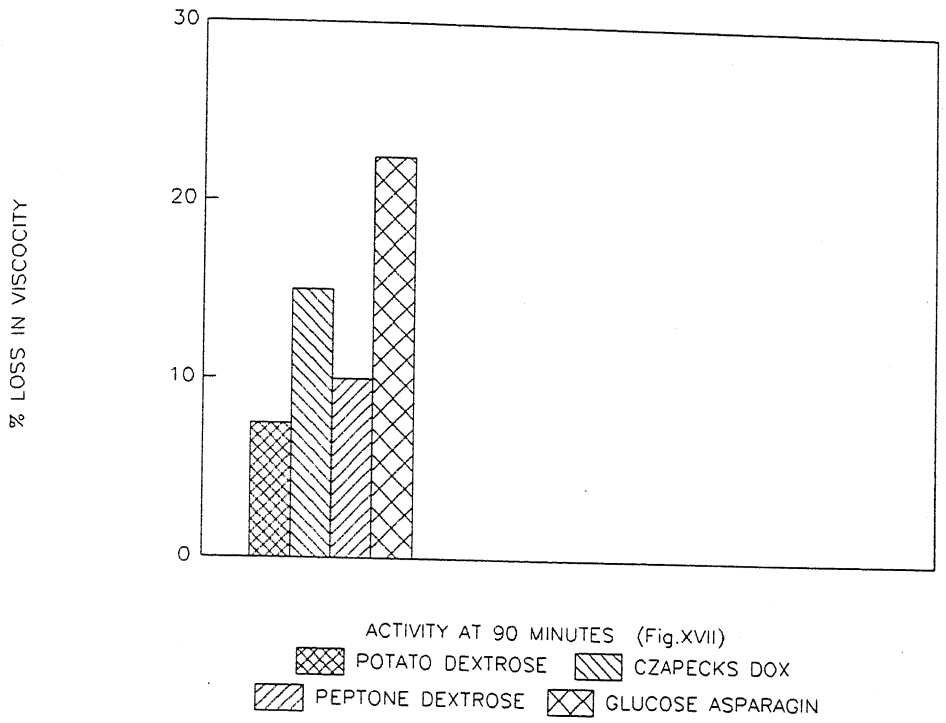
% LOSS IN VISCOCITY

TIME IN MINUTES (Fig.XVI)

□ POTATO DEXTROSE + CZAPECKS DOX

◇ PEPTONE DEXTROSE △ GLUCOSE ASPARAGIN

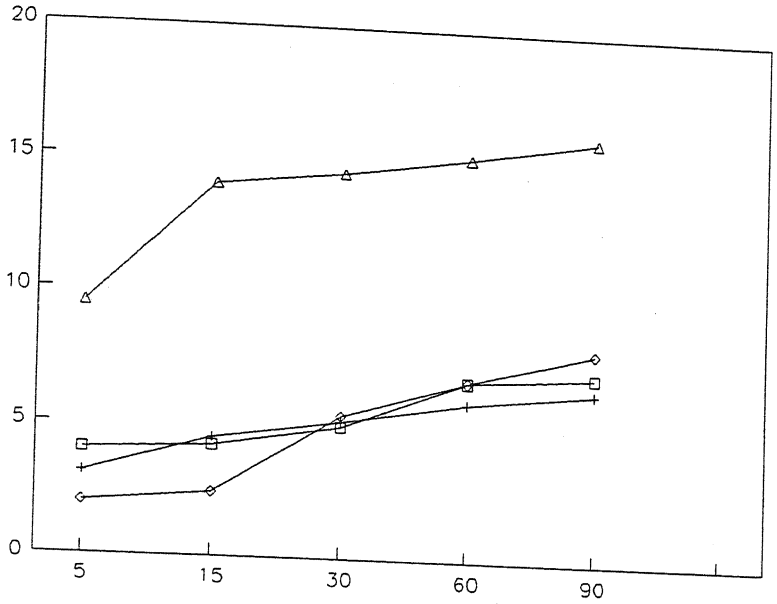
PMG ACTIVITY AFTER 10 DAYS OF INCUBATION



ACTIVITY AT 90 MINUTES (Fig.XVII)

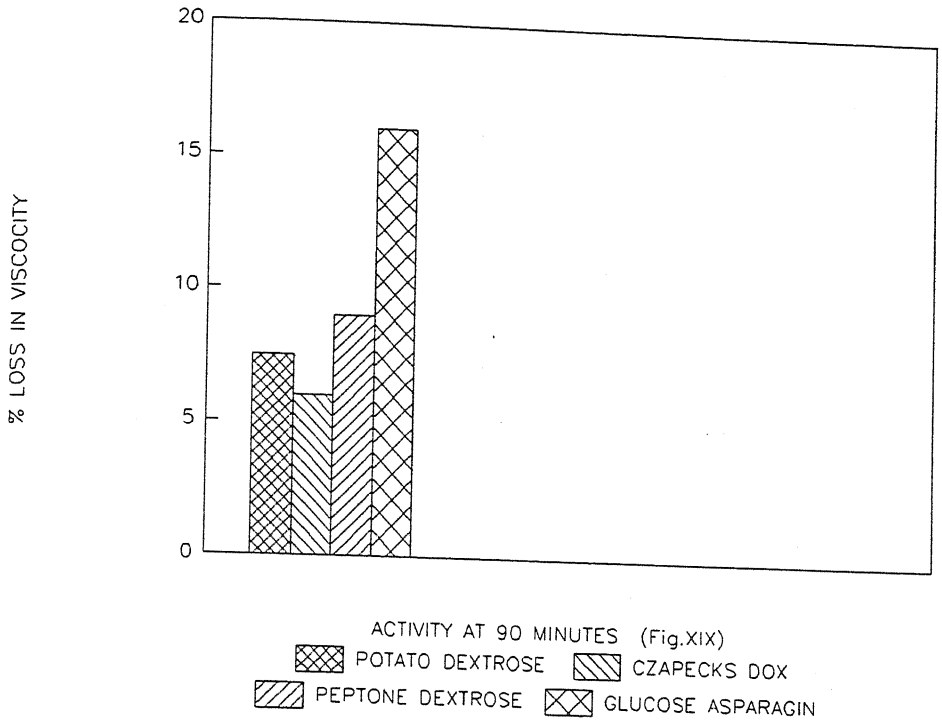
POTATO DEXTROSE
CZAPECKS DOX
PEPTONE DEXTROSE
GLUCOSE ASPARAGIN

PGTE ACTIVITY AFTER 5 DAYS OF INCUBATION



(Fig.XVIII)

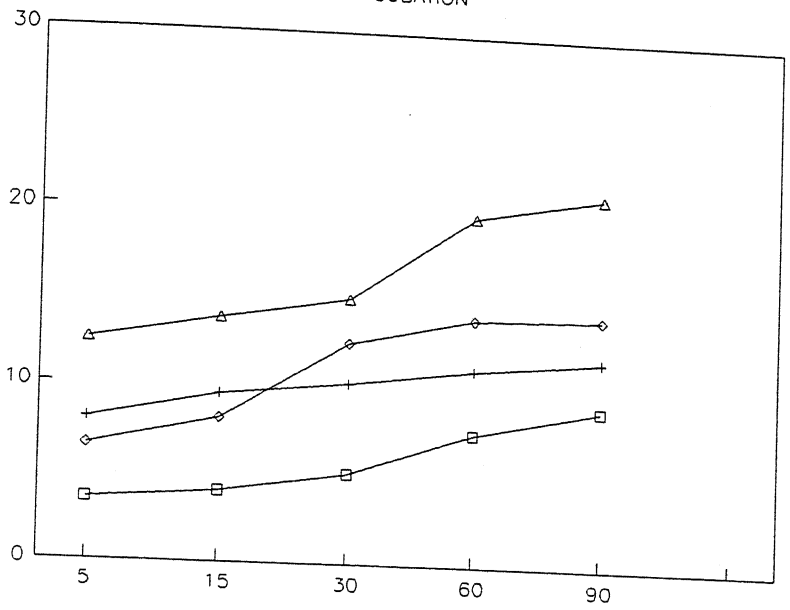
PGTE ACTIVITY AFTER 5 DAYS OF INCUBATION



ACTIVITY AT 90 MINUTES (Fig.XIX)

POTATO DEXTROSE
CZAPECKS DOX
PEPTONE DEXTROSE
GLUCOSE ASPARAGIN

PGTE ACTIVITY AFTER 10 DAYS OF INCUBATION



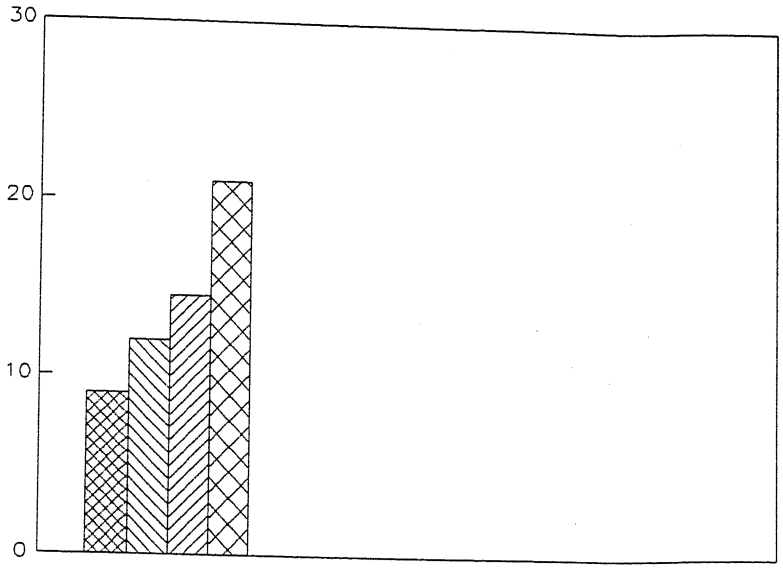
% LOSS IN VISCOCITY

TIME IN MINUTES (Fig.XX)

□ POTATO DEXTROSE + CZAPECKS DOX

◇ PEPTONE DEXTROSE △ GLUCOSE ASPARAGIN

PGTE ACTIVITY AFTER 10 DAYS OF INCUBATION

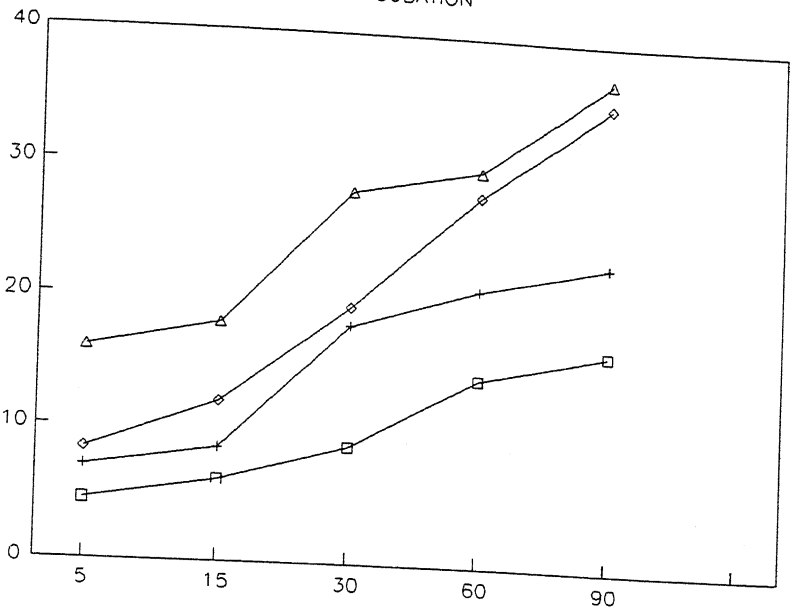


ACTIVITY AT 90 MINUTES (Fig.XXI)

POTATO DEXTROSE CZAPECKS DOX

PEPTONE DEXTROSE GLUCOSE ASPARAGIN

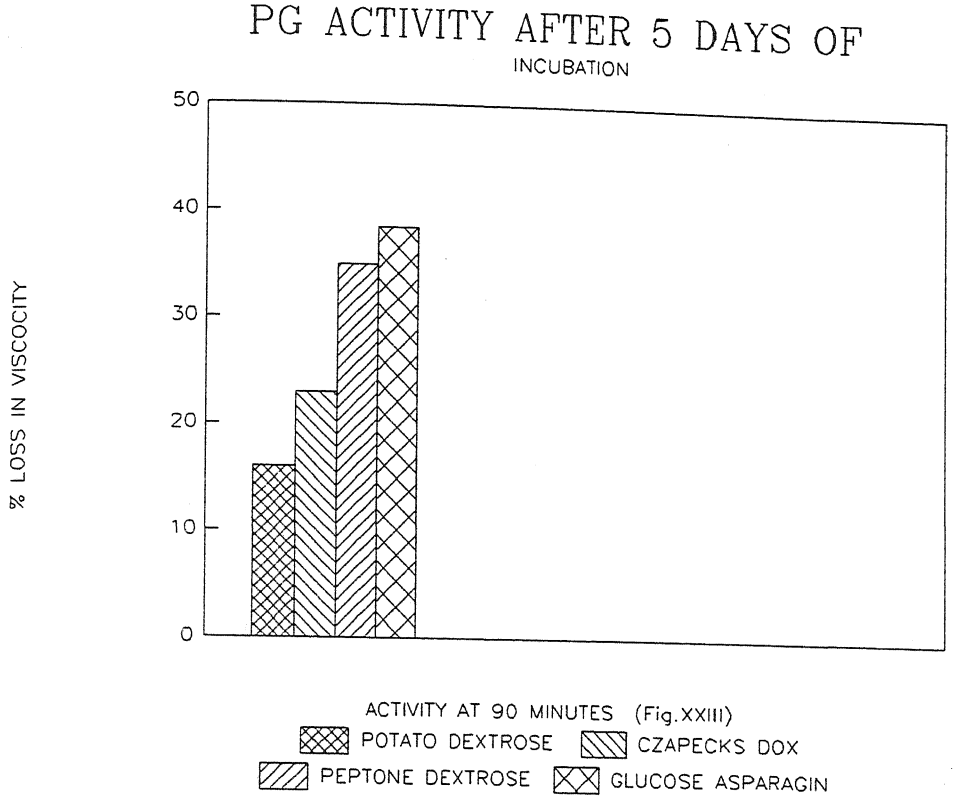
PG ACTIVITY AFTER 5 DAYS OF INCUBATION



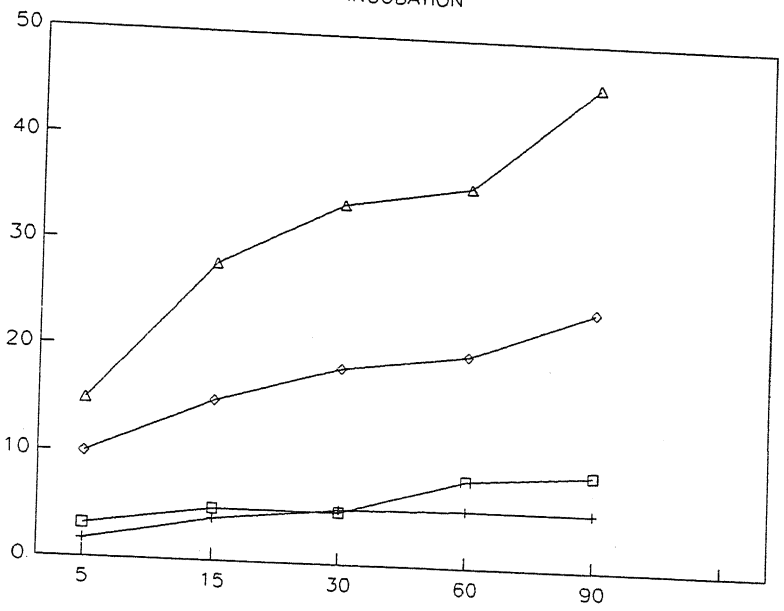
(Fig.XXII)

□ POTATO DEXTROSE + CZAPECKS DOX

◇ PEPTONE DEXTROSE △ GLUCOSE ASPARAGIN


PG ACTIVITY AFTER 5 DAYS OF
INCUBATION
% LOSS IN VISCOCITY
50
40
30
20
10
0
ACTIVITY AT 90 MINUTES (Fig.XXIII)
POTATO DEXTROSE
CZAPECKS DOX
PEPTONE DEXTROSE
GLUCOSE ASPARAGIN

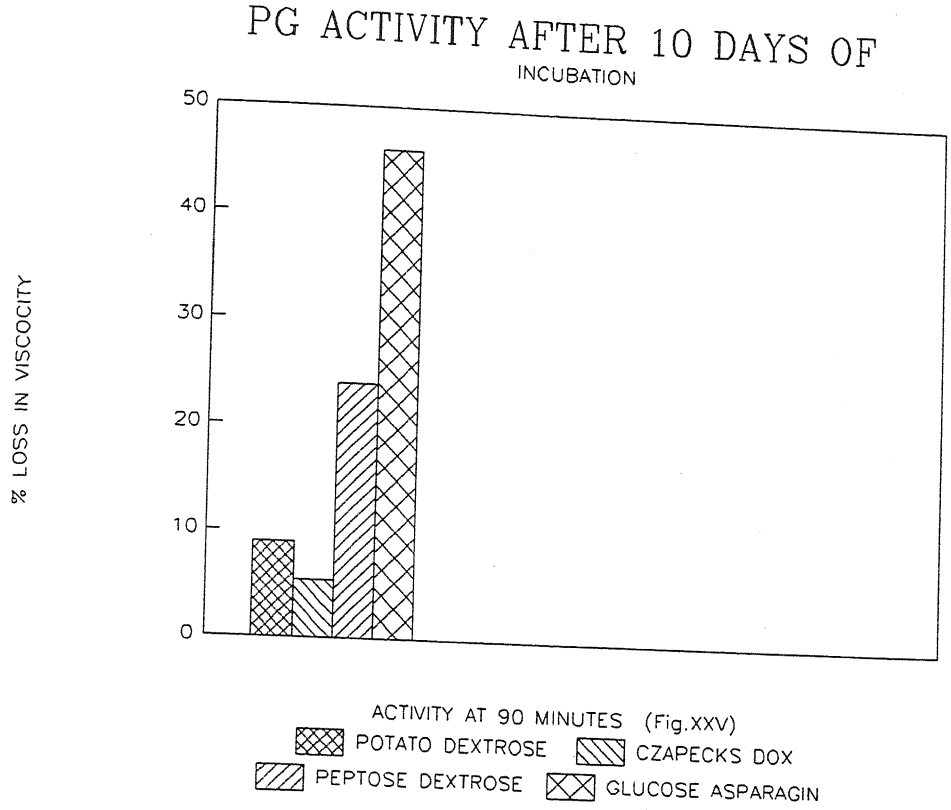
PG ACTIVITY AFTER 10 DAYS OF INCUBATION

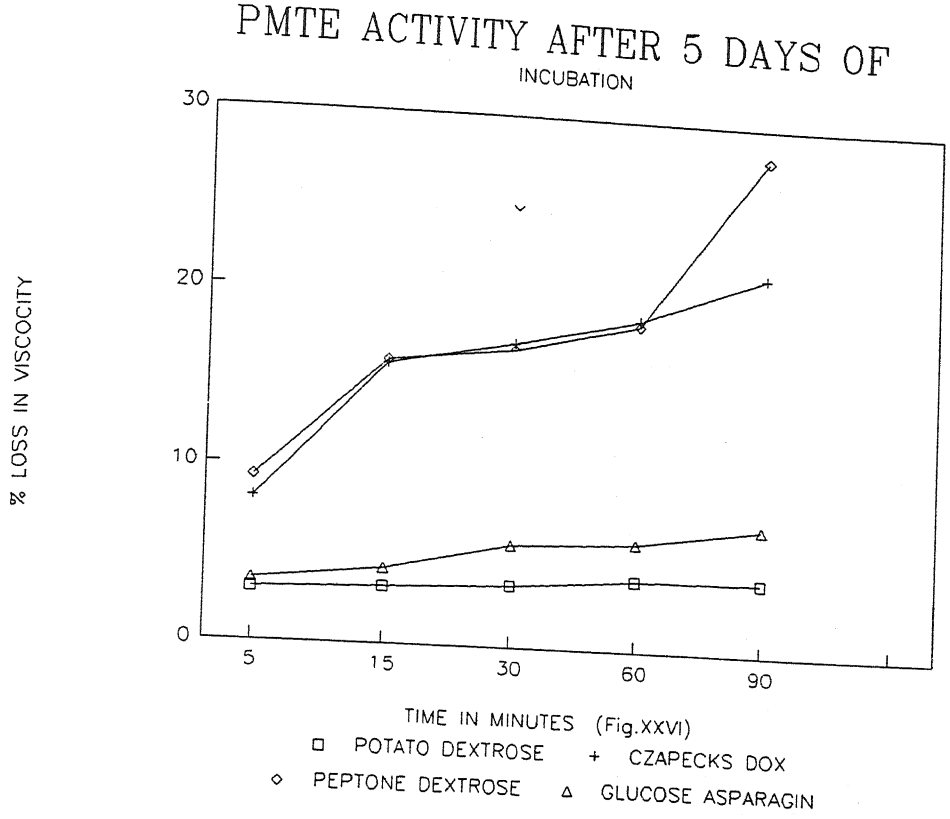


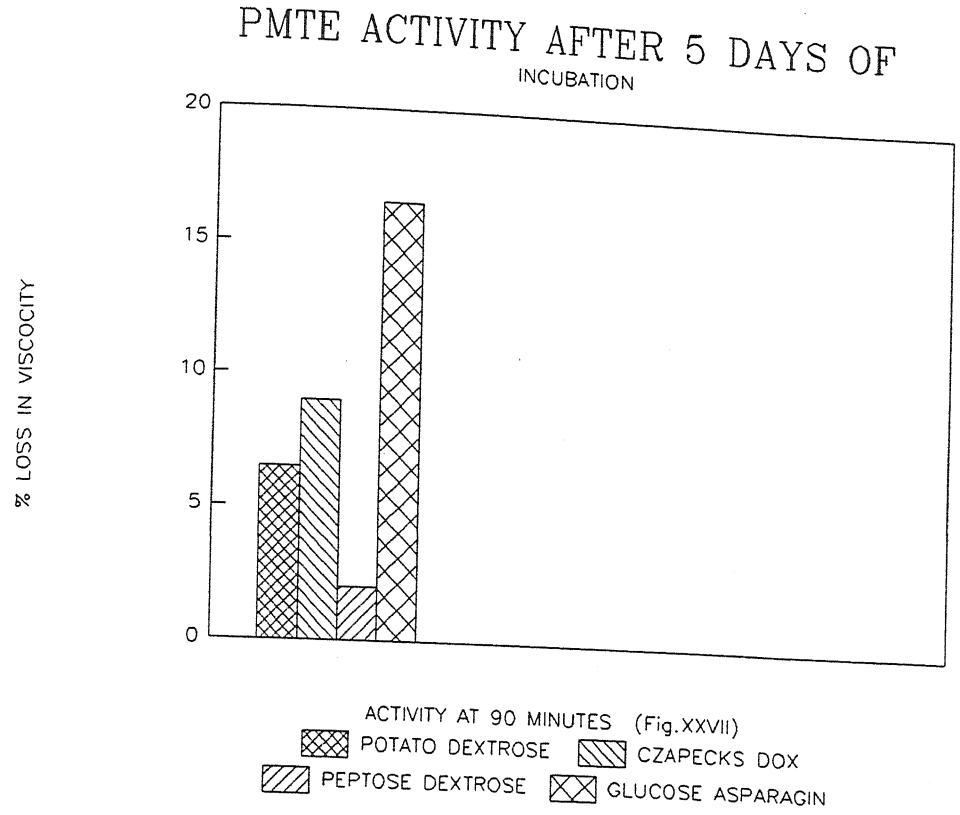
(Fig.XXIV)

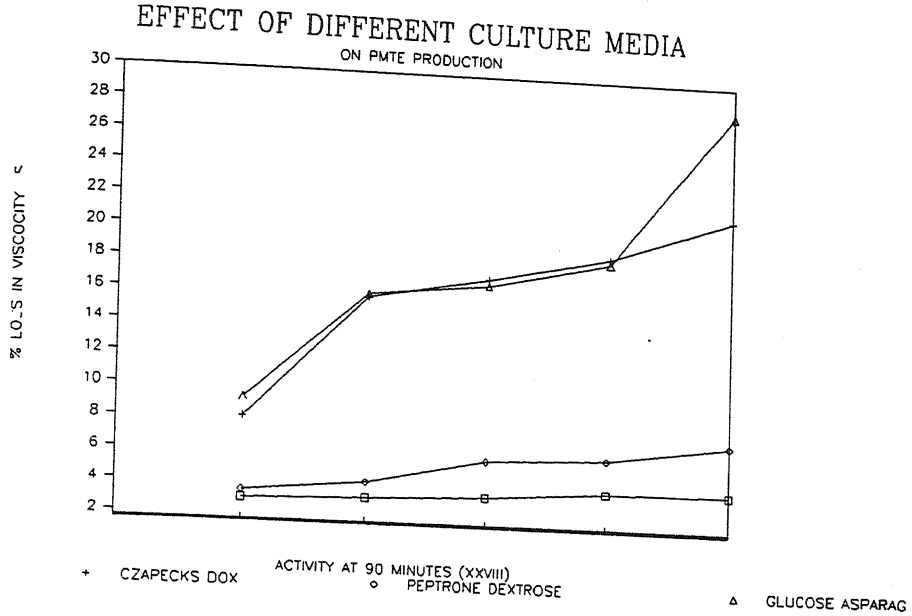
□ POTATO DEXTROSE + CZAPECKS DOX

◇ PEPTONE DEXTROSE △ GLUCOSE ASPARAGIN

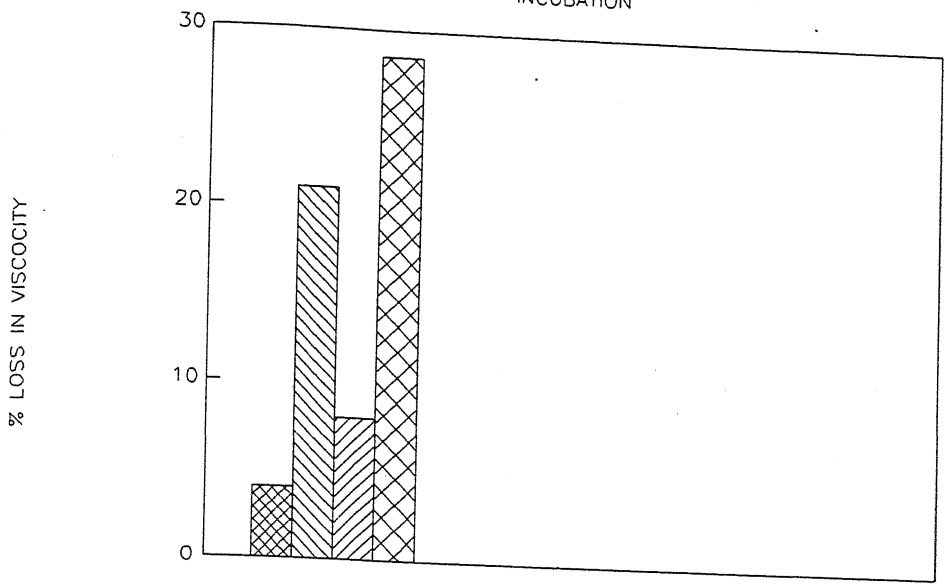

PG ACTIVITY AFTER 10 DAYS OF
INCUBATION
% LOSS IN VISCOCITY
50
40
30
20
10
0
ACTIVITY AT 90 MINUTES (Fig.XXV)
POTATO DEXTROSE
CZAPECKS DOX
PEPTOSE DEXTROSE
GLUCOSE ASPARAGIN


PMTE ACTIVITY AFTER 5 DAYS OF INCUBATION
% LOSS IN VISCOCITY
30
20
10
0
5
15
30
60
90
TIME IN MINUTES (Fig.XXVI)
□ POTATO DEXTROSE
+ CZAPECKS DOX
◇ PEPTONE DEXTROSE
△ GLUCOSE ASPARAGIN


PMTE ACTIVITY AFTER 5 DAYS OF
INCUBATION
% LOSS IN VISCOCITY
20
15
10
5
0
ACTIVITY AT 90 MINUTES (Fig.XXVII)
POTATO DEXTROSE
CZAPECKS DOX
PEPTOSE DEXTROSE
GLUCOSE ASPARAGIN


EFFECT OF DIFFERENT CULTURE MEDIA
ON PMTE PRODUCTION
% LO-S IN VISCOCITY
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
ACTIVITY AT 90 MINUTES (XXVIII)
□ POTATO DEXTROSE
+ CZAPECKS DOX
◇ PEPTRONE DEXTROSE
△ GLUCOSE ASPARAG

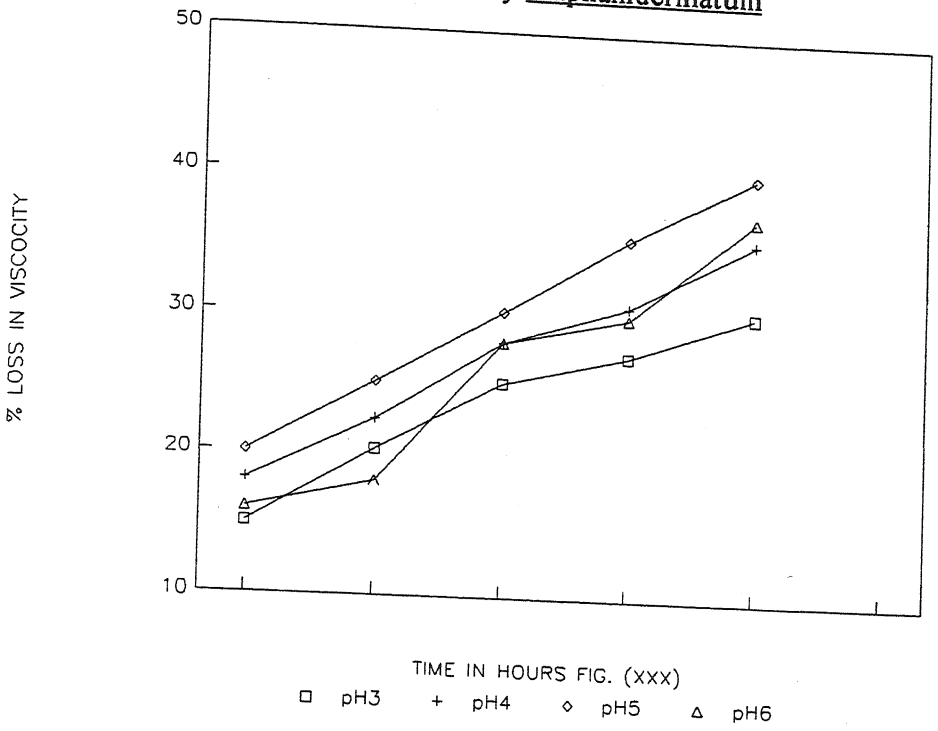
PMTE ACTIVITY AFTER 10 DAYS OF INCUBATION



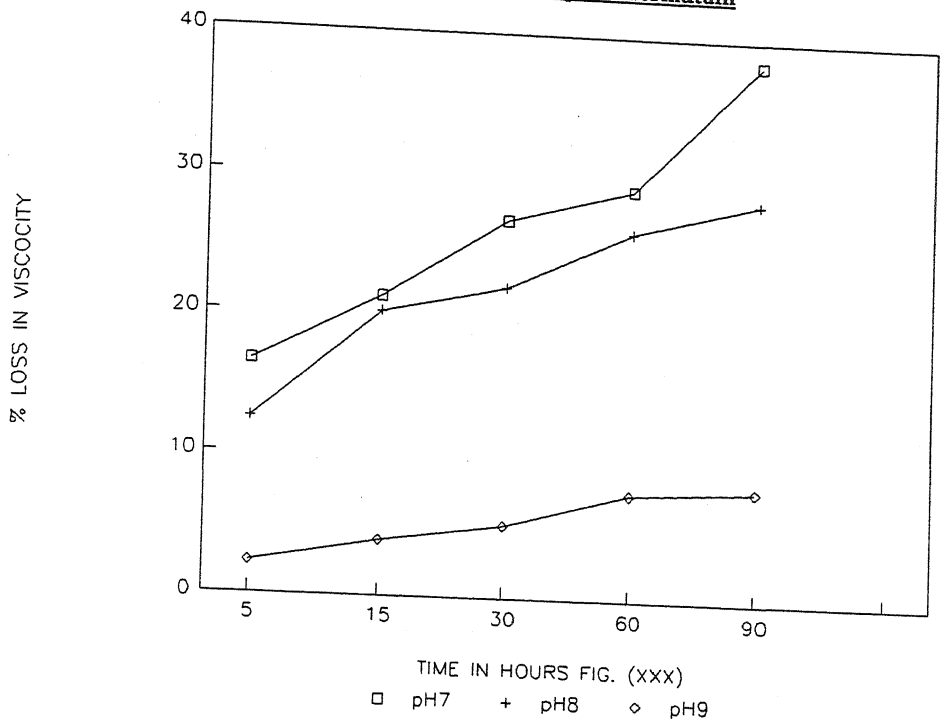
ACTIVITY AT 90 MINUTES (Fig.XXIX)

POTATO DEXTROSE
CZAPECKS DOX
PEPTOSE DEXTROSE
GLUCOSE ASPARAGIN

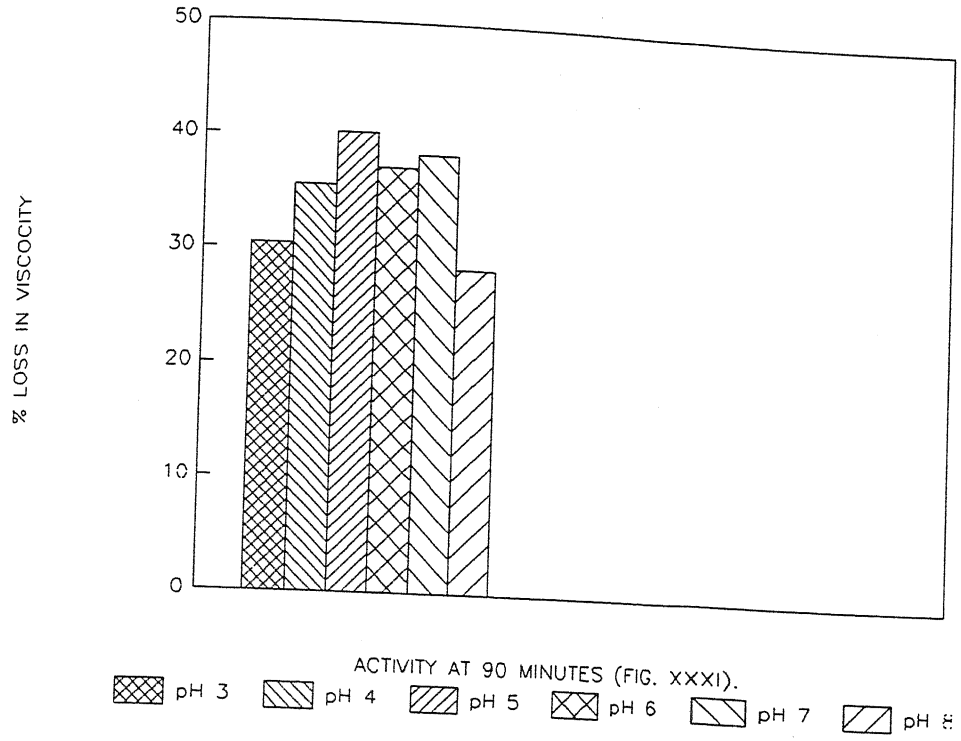
EFFECT OF pH ON POLYGALACTRONASE
Production by P.aphanidermatum



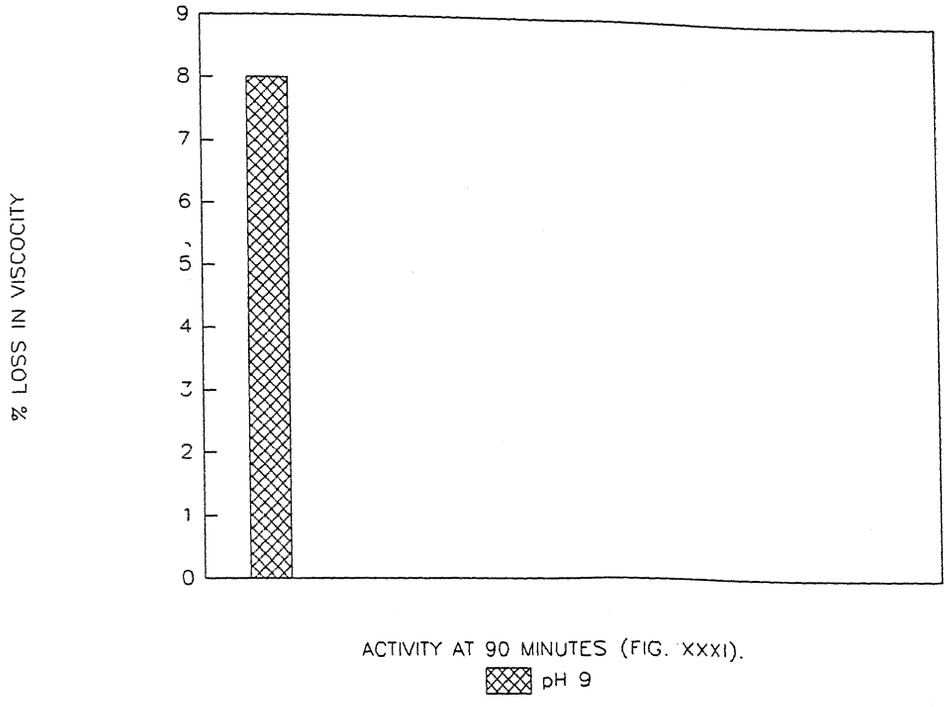
EFFECT OF pH ON POLYGALACTRONASE PRODUCTION by P. aphanidermatum



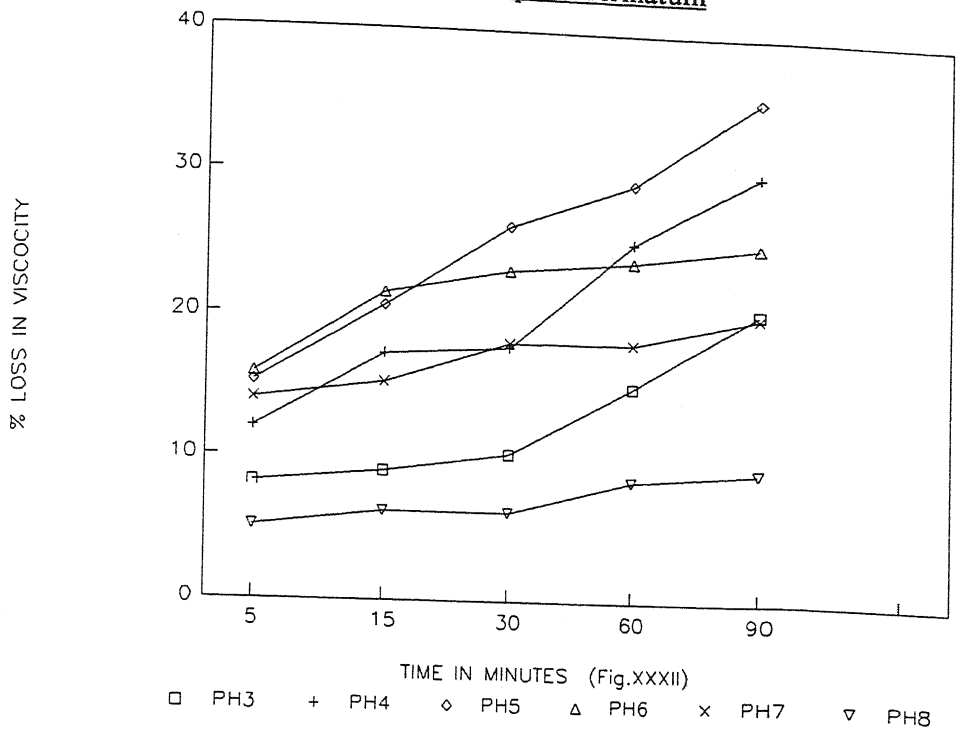
EFFECT OF pH ON PG PRODUCTION



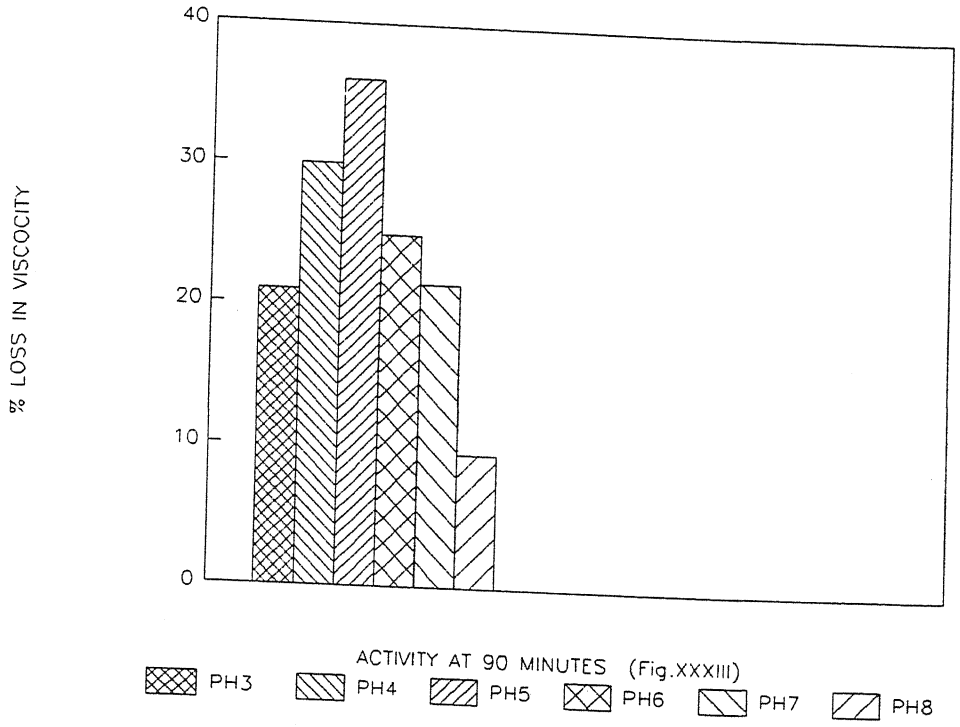
EFFECT OF pH ON PG PRODUCTION



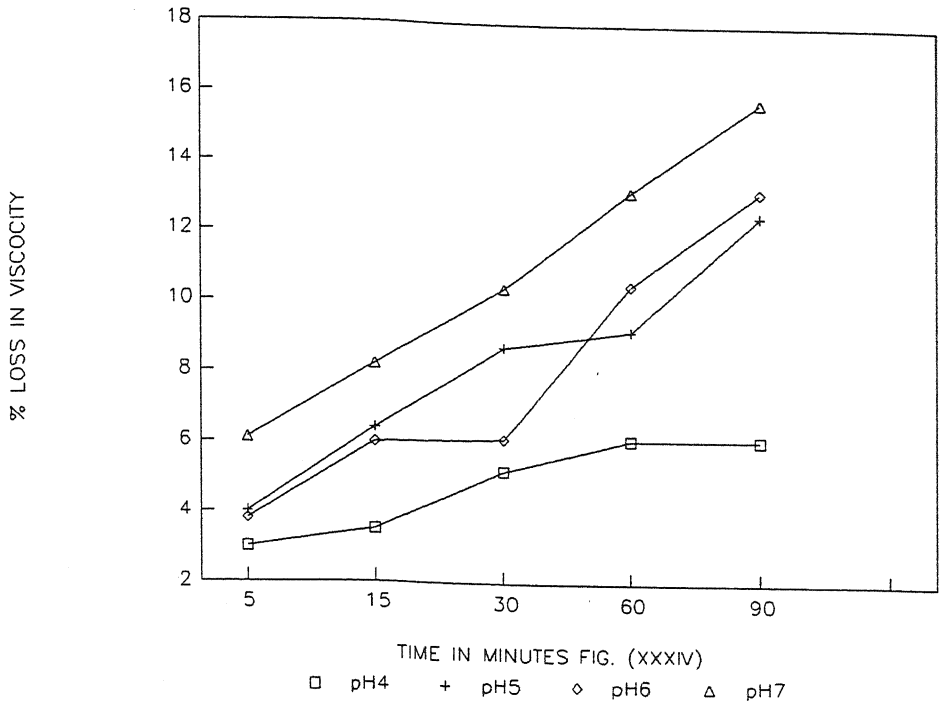
EFFECT OF pH ON PMG PRODUCTION
by P.aphanidermatum



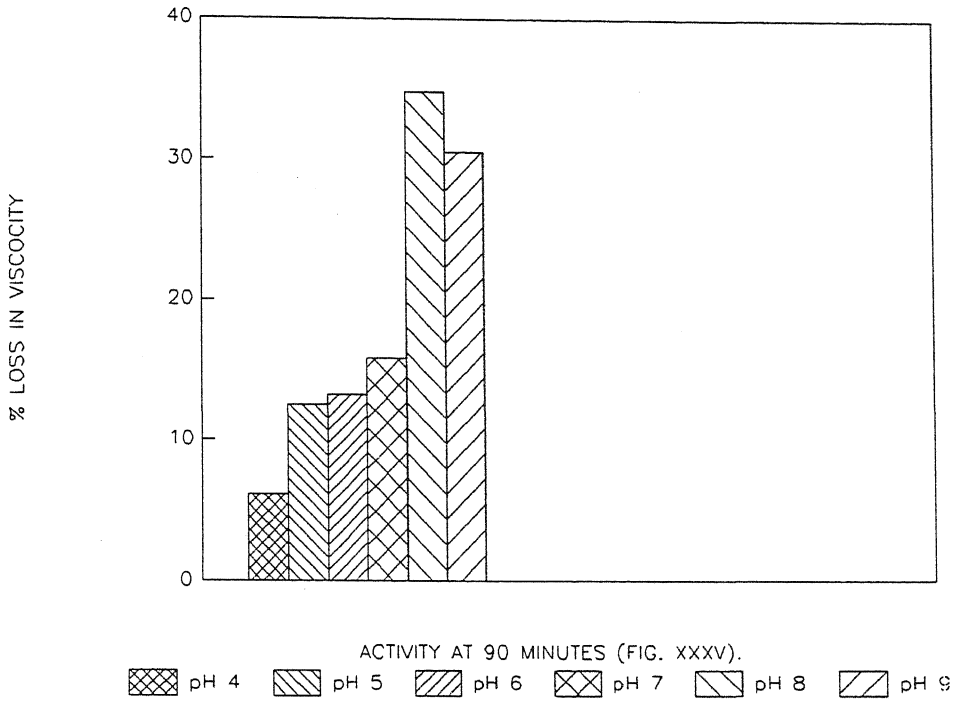
EFFECT OF pH ON PMG PRODUCTION



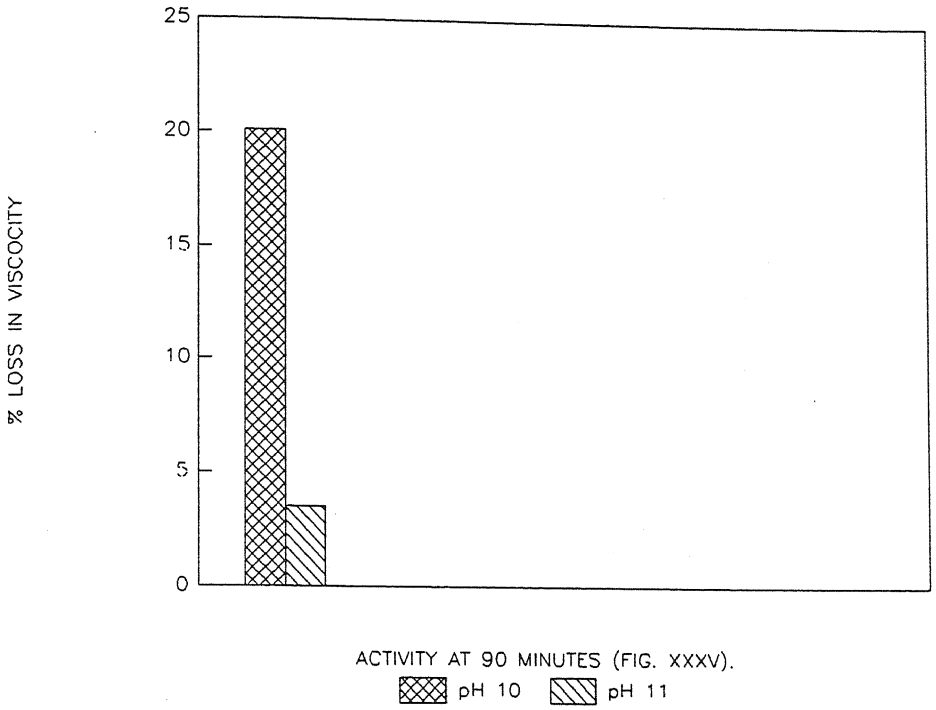
EFFECT OF pH ON POLYGALACTRONASE TRANSELIMINASE PRODUCTION by P. aphanidermatum



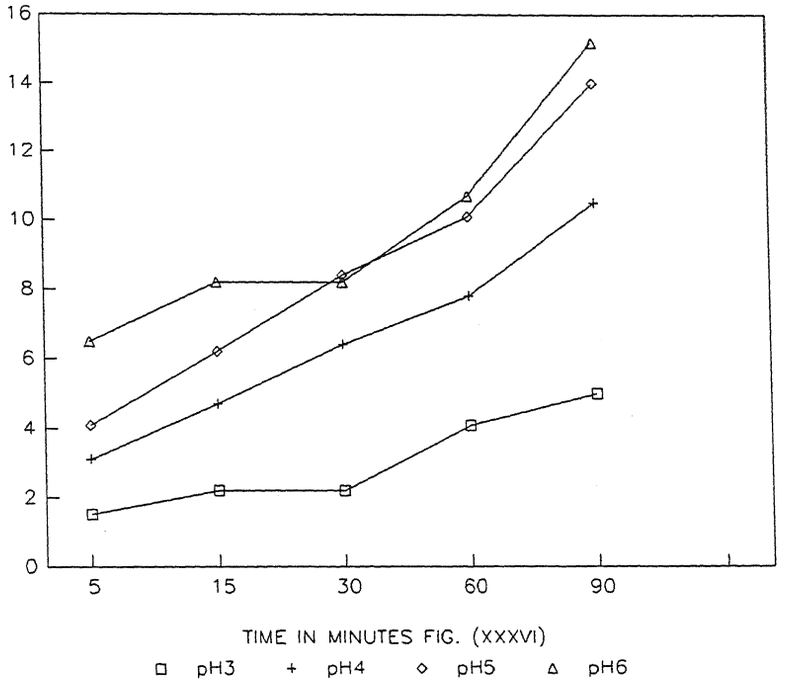
EFFECT OF pH ON PGTE PRODUCTION



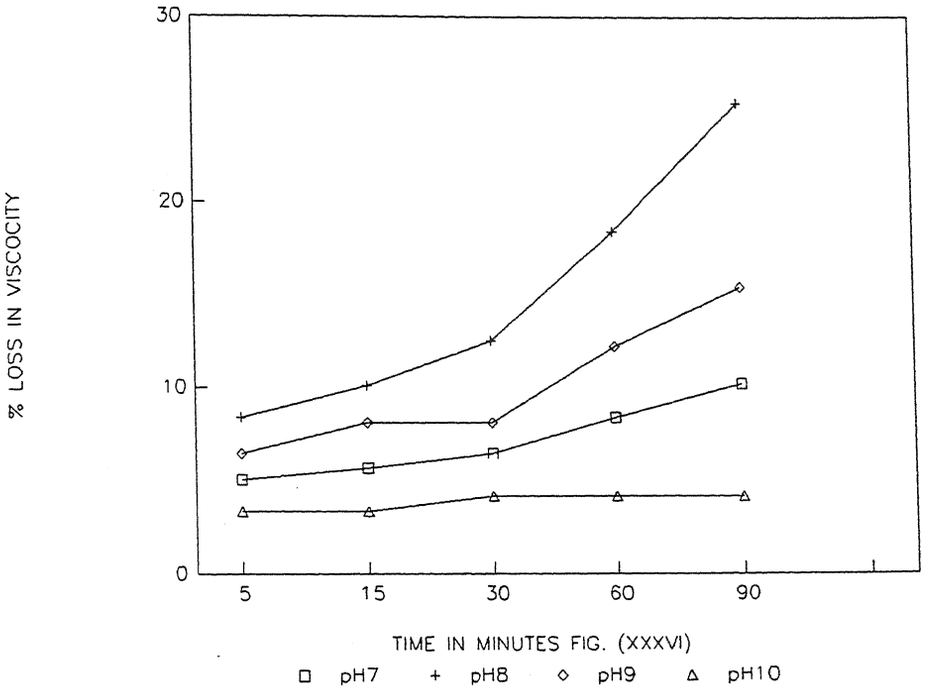
EFFECT OF pH ON PGTE PRODUCTION by P. aphanidermatum



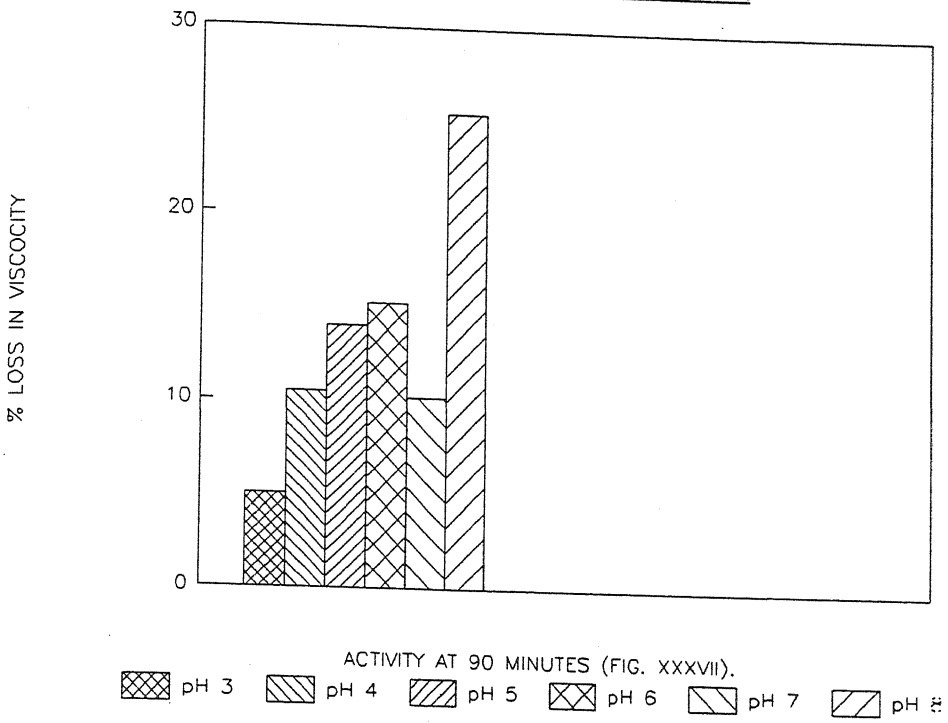
EFFECT OF pH ON POLYGALACTRONASE PRODUCTION by P. aphanidermatum



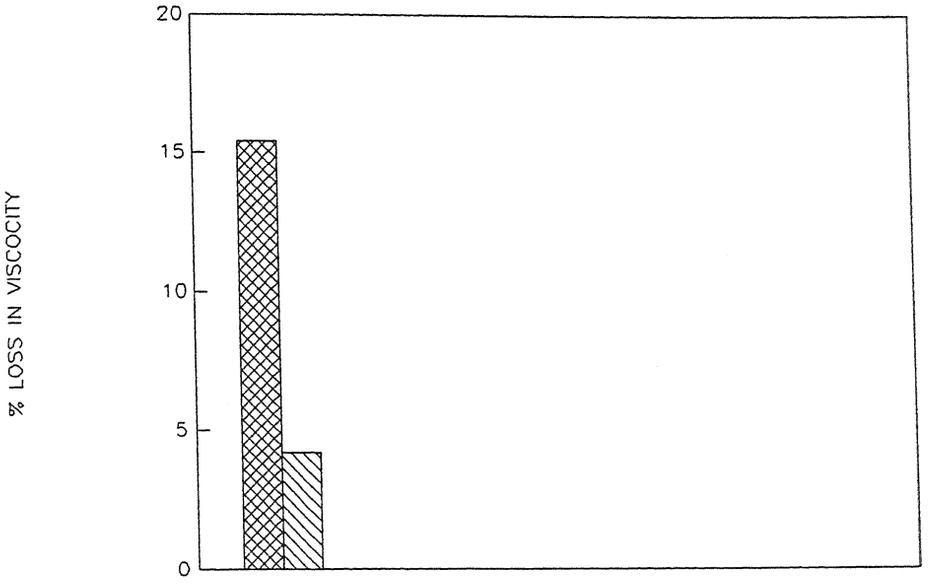
EFFECT OF pH ON POLYGALACTRONASE PRODUCTION by P. aphanidermatum



EFFECT OF pH ON PMTE PRODUCTION by P. aphanidermatum



EFFECT OF pH ON PMTE PRODUCTION by P. aphanidermatum



ACTIVITY AT 90 MINUTES (FIG. XXXVII).

pH 9 pH 10

CHAPTER XIX

DISCUSSION AND CONCLUSION

The production of particular enzyme by the pathogen is frequently determined by the environmental condition. The various conditions include the composition of the medium, the nature of carbon source,incubation period,pH of the medium and so on. Therefore to determine the effect of various cultural conditions the present studies were made and the results obtained in the previous chapter are discussed below.

EFFECT OF CULTURAL MEDIA

Among the various cultural media tested Glucose Asparagin was found to be the most favourable than others.For most ofthe enzymes however Proto pectinase activity was more in Potato Dextrose. As compared to the other medium Czapecks Dox was the next followed by Peptone Dextrose and Glucose Asparagin. Here Glucose Asparagin is least favoured. Five days old culture gave the best result, Table XIV PME activity was not found in vitro in all the mediums tested either after 5 days or 10 days of incubation. These observations are similar to those of Winstead and McComb (1961) and Indrasenan and Paily (1982) P.aphanidermatum produced varying amount of PG, PMG,PGTE and PMTE in different culture media.Glucose Asparagin was found to be most favourable than others for the production of PG while maximum PG activity was obtained on 5 days Incubation period. Table XVI , Fig. XXII & XXV.

With regard to PMG it was observed that Czapecks Dox was more favourable on 5 days incubation period and Glucose Asparagin was more favourable on 10 days of incubation than the other medium tested. Table XV, Fig. XIV to XVII.

Regarding the effect of various culture media on the production of 2 transeliminases it was found that Glucose Asparagin was more favourable as compared to the other medium.-

-Maximum activity was observed in both the cases after 10 days of incubation Table XVII & XVIII Fig. XVIII to XXI & Fig. XXVI to XXIX . On the basis of overall results it may be stated that there is most efficient production of Glycosidases than transeliminases. On account of the number of factors and mechanisms involved simultaneously in enzyme secretion, it is difficult to reach on a definite conclusion with regard to any specific factors. However on the basis of the results in the present study it may be mentioned that overall performance was best and 10 days of incubation could be considered to be optimum for

Transeliminases and 5 days for Glycosidases. During the course of study it was also observed that however mycelial growth was best on Peptone Dextrose but generally enzyme production was less. Thus there can be correlation between the mycelial growth and enzyme production. Glucose appears to be a better carbon source as compared to Dextrose and Sucrose, for enzyme production by P.aphanidermatum. Similar enzymological study using the various culture media for production of various Pectolytic enzyme have been carried out by Ali (1970), Rai (1971),

Indrasenan and Paily(1982) and others. They have also observed that different culture media exert variable effect on the Pectic enzyme production in culture. The variation may be probably due to various cultural conditions. including cultural media, their constituent, pH and incubation period.

Effect of pH

According to Sher wood (1966) Bateman(1966) & Hancock(1966) pH change of the media play a significant role in Pectic enzyme production by the pathogenin vitro.With regards to the effect of the pH it was observed that the production of Glycosidases was favoured by the acitic range while alkaline pH supported the synthesis of transeliminases .Table XIX , XX, XXI, XXII & Fig. XXX to XXXVII. Sher wood (1966),Hancock(1966) Ali(1970), Rai (1971) have also come to the same conclusions.

PART II

SECTION 'A'

SUB SECTION 'c'

CHAPTER XX

INTRODUCTION

IN VITRO STUDIES OF PECTIC ENZYMES

IN PRESENCE OF GROWTH REGULATORS AND FUNGICIDES

The pectic enzyme produced by pathogenic fungi are variously influenced by a number of substances present in the medium A number of substances if added to the medium also influence the pectic enzyme production.Gupta (1956), described the effect of cultural conditions on the secretion of pectic enzymes by P.debaryanum..Enzyme secretion was increased when NaCl was added and the medium contains Glucose as the Carbon source. Similarly Sherwood (1966) and Gupta (1956) incorporated Sodium Chloride in synthetic medium which which stimulated the activity of P.debaryanum. Glucose, Fructose and Mannose were also found to be equally suitable for the growth & enzyme production . Sucrose gave good growth but poor enzyme production. Galactose gave very poor growth and negligible enzyme production. Damle (1952) working with Ashour's Medium found that active secretion of enzyme was not obtained unless the glucose constituent was autoclaved with Phosphate(K3PO4 & K2PO4).

Auto claving with acid phosphate was in effective even when the medium was subsequently adjusted to a pH favourable to the growth of the fungus . The enzyme production and its influence on various hexoses studied by Gupta (1956) for Pythium debaryanum. Fernando (1937) studied the effect of Nutrient medium on the secretion and properties of Pectinase. Winstead and Mccomb (1961) observe the effect of CMC, Sodium polypectate and macerated filter paper on the Pectic Enzyme production by P.aphanidermatum.

Thus it is clear that various substances in the environment of plant pathogen regulate the production of the Poly sacchride degrading enzymes. Besides carbo hydrates other compounds like plant Growth Regulators and Fungicides are also known to control the production of various Pectic Enzymes by pathogenesis.

Number of growth regulators control the synthesis of enzymes.These were also found to be involved in several plant diseases caused by the fungal pathogens. The studies concerning the effect on the synthesis of Pectic Enzymes were however were little. Indole Acetic Acids has been found to be a binding agent that regulates the amount of bound and free PME in plant cell wall.Glazious (1961) . Although not much work has been done on the effect of plant growth regulators on the production and activity of Pectic Enzyme by fungal pathogens

Their controlling influence or repressions of Pectic Enzyme synthesis cannot be ruled out.Therefore,a study in this direction may prove to be quite rewarding,thus included in this chapter. These substances influence the growth of pathogen on one hand and,inhibit the growth of pathogen on the other hand. Thus it appears to be useful for present investigation.

Fungicides are organic compounds which have a direct action against the fungal pathogens. The fungi toxicity in some cases have been attributed to their potentiality for disrupting or blocking certain respiratory enzymes.Sisler and Cox (1960),Foot et.al.(1949), Horsefall (1956), Grover and Moore (1962),Grover,Goyal and Malhotra (1973-74) have also reported that fungicides inhibit production and activity of various pectolytic enzymes.

Grover (1961) while working with Scleroceium ,sclerotiarum and Botrytisalli demonstrated that the pectolytic enzyme activity in the filtrate of both fungi was reduced to the minimum when Phalten and Difolatan were incorporated in the medium.

Therefore fungicides could be used for inhibitory effect of enzyme activity. Their selection could be based on the inhibitory effect of fungal growth followed by test in laboratories. Sugunakara Reddy, Rao, et.al.(1979) studied the fungicides tolerance of fungi and found that the quality of inoculum ,nature of inoculum, age of inoculum, pH of the medium and concentration of the medium respond indifferently to the fungal effect against the plant pathagens.Ramraj and Vidya shekaran (1986) studied the effects of fungicides on the Pectolytic enzymes of P.parasitica .Some of these fungicides caused inactivation of enzymes at lower concentration(50ppm) But some where also effective at higher concentration (250 ppm) Goyal & Malhotra (1973-74) studied the effect of some fungicides on Pectolytic and Cellulolytic Enzyme production of Rhizoctonia bataticola.They found Ceresan,Thiram and Brassicol to be most effective and reduced the enzyme production in the culture media.It was interesting to note inhibition or inactivation of pectolytic and cellulotic enzymes could be correlated with the efficiency of fungicides to root rot diseases and eliminating the seed born infection.From the above account it appears that some fungicides which are selected or rejected after lab treatment on the basis of fungal growth might be useless or may prove to be better than those that are selected. Based on the above background the present study was planned in which the effect of various plant growth regulators and certain fungicides on the production of Pectic enzymes by P.aphanidermatum invitro have been studied.

CHAPTER XXI

EXPERIMENTAL

1 EFFECT OF PLANT GROWTH REGULATORS

Five growth regulators were used for this study. They were

1. INDOLE ACETIC ACID (IAA)

2. INDOLE BUTYRIC ACID (IBA)

3. INDOLE PROPIONIC ACID (IPA)

4. GIBBERELIC ACID

5. KINETIN

The sterilized Glucose Asparaginemedium was selected for the present study since most of the enzyme give better results. The above sterilized medium before inoculation was supplemented with 10 ppm. concentration of plant growth regulators. Table XXIII, XXIV, XXV & XVI & Fig . XXXVII - XLV.

2. EFFECT OF FUNGICIDES

The following fungicides in a concentration of .5% were added in the Glucose Asparaginemedium before sterilization.

1. Thiram

2. Brassicol

3. Blitane

4. Captane

The medium were finally inoculated with P.aphanidermatum and incubated for 5days at 26 ± 2° C .The extractionof — enzyme preparation and the method of enzyme assay were the same as described earlier in the previous chapter. Table XXVII, XXVIII, XXIX, XXX & Fig. XLVI - LIII.

The enzymatic studies were restricted to PG, PMG, PGTE, and PMTE. Protopectinase and PME were not selected for these investigations because of their insignificant development during the studies in the previous chapters.

Glucose Asparaginemedium without growth regulators orfungicides was taken as control. Triplicates were taken in each case.

CHAPTER XXII

RESULTS AND OBSERVATIONS

1. EFFECT OF PLANT GROWTH REGULATORS ON PECTOLYTIC ENZYME PRODUCTION

Effect of IAA, IBA, IPA, Gibberelic acid & Kinetin were studied on the production of PG. PMG, PGTE, PMTE. The date obtained are given in the table XXIII, XXIV, XXV, XXVI, & Fig. XXXVII-XLV.

From the data it is revealed that both Glycosidases and transeliminases were considerably affected by various plant growth substances. None of the hormones increased the synthesis of any of the pectic enzyme studied as in no case the activity was greater than that of the control . In all the cases when growth regulatory substance was added there was lower enzyme activity than that of the control was observed . Comparatively PG was less affected as compared to PMG, PMTE, and PGTE. Out of the 5 growth regulators IPA & Kinetin caused a substantial decrease in the production of Poly Galactronase. Gibberelic acid however did not affect the synthesis of this enzyme in culture because the enzyme activity with this hormone was more or less similar with that of control. Table XXIII & Fig. XXX,XXXIX.

The production of another glycosidases i.e.,. PMG was strongly suppressed by majority of plant growth regulatory substance. Among these Kinetin and IPA were more effective than others. IAA was least affected in this case. Table XXIV & Fig XL, XLI.

The formation of both transeliminases(PGTE & PMTE) were generally decreased in presence of all growth regulators. PMTE comparatively was more affected than PGTE. In PGTE synthesis,Gibberelic acid and Kinetin were more effective in decreasing the production of the enzyme. IPA however, surprisingly was least effected in this case. Table XXV Fig XLII, XLIII.

In PMTE. synthesis,IBA and Gibberelic acid were more effective than others. IAA again failed to produce a significant effect on this enzyme. Table XXVI Fig. XLIV,XLV. The over all study show that Kinetin was effective in majority of the enzyme studied. During the course of study the author noticed that the mycelial growth was less in the media inoculated with the hormones as compared to that of the control. Mycelial growth obtained individually and when compared with the enzyme production for each growth regulatory substance, it was found,that there could be no correlation between the fungal growth and the enzyme secretion of P.aphanidermatum in the presence of various plant growth regulators.

EFFECT OF FUNGICIDES

Effect of Thiram, Brassicol, Blitane, and Captane on the production of PG, PMG, PGTE, PMTE was studied . The datas are given in Table XXVII,XXVIII,XXIX,XXX. & Fig. XLVI - LIII.

From the datas it is observed that the Pectic enzymes secretion by Pythium aphanidermatumwas inhibited variedly by Thiram, Captane ,Blitane and Brassicol respectively. These were highly toxic to the pathogen and thus it could synthesize insignificant amount of PG. Brassicol and Blitane were comparatively less toxic to enzyme production and PMG synthesis was also inhibited by Thiram ,Captane,Blitane,and Brassicol .But,were not so effective as in case of PG. Synthesis of PMG was least affected as compared to PG, ,PMTE,PGTE,synthesis which was fully inhibited by Thiram and Captane. In Blitane and Brassicol Pythium aphanidermatum did manage to develop some PMTE but in insignificant amount.

Among the four Pectolytic enzymes (PG,PMG,PGTE and PMTE) PG and PMTE were more sensitive to fungicides.

From the over all research in the table XXVII,XXVIII,XXX, Fig. XLVI-LIII .It is evident that Thiram is the most effective fungicide followed by the Captane in reducing the production of Pectolytic Enzymes by P.aphanidermatum.. Among the Pectic Enzymes the production PG and PMTE was most adversely affected. .

TABLE XVIII

EFFECT OF PLANT GROWTH REGULATORS ON THE PRODUCTION OF POLYGALACTRONASE BY P.aphanidermatum

S No	PLANT GROWTH REGULATORS	CONCENTRATION ppm.	% LOSS IN VISCOCITY AFTER MINUTES				
			5	15	30	60	90
1.	Indole Acetic acid	10	8.2	9.8	14.2	20.1	23.3
2.	Indole Butyric acid	10	5.4	9.2	14.2	20.5	22.5
3.	Indole Propionic acid	10	4.1	7.4	12.3	14.5	18.1
4.	Gibberellic acid	10	15.1	19.2	30.1	32.4	36.3
5.	Kinetin	10	5.8	8.2	14.3	15.6	19.1
6.	CONTROL	10	16.0	18.0	28.1	30.0	37.2

TABLE XXIV

EFFECT OF PLANT GROWTH REGULATORS ON THE PRODUCTION OF PECTIN METHYL GALACTRONASE BY P.aphanidermatum

S NO	PLANT GROWTH REGULATORS	CONCENTRATION ppm	% LOSS IN VISCOCITY AFTER MINUTES				
			5	15	30	60	90
1.	I A A	10	5.1	5.5	9.3	10.7	12.2
2.	I B A	10	1.2	3.4	3.9	4.3	6.4
3.	I P A	10	1.5	1.6	4.3	5.2	5.9
4.	GIBBERELLIC ACID	10	2.1	2.1	3.8	5.5	6.1
5.	KINETIN	10	1.1	1.3	3.5	4.8	5.5
6.	CONTROL	10	4.0	6.9	10.1	11.3	15

TABLE XXV

EFFECT OF PLANT GROWTH REGULATORS ON THE PRODUCTION OF POLYSALACTRONASE BY P.aphanidermatum

SNO.	PLANT GROWTH REGULATORS	CONCENTRATION ppm.	% LOSS IN VISCOCITY AFTER MINUTES				
			5	15	30	60	90
1.	Indole Acetic acid	10	1.1	2.5	5.4	7.8	8.9
2.	Indole Butyric acid	10	3.2	5.3	5.5	6.4	9.2
3.	Indole Propionic acid	10	4.1	5.9	6.2	7.1	10.1
4.	Gibberellic acid	10	2.4	4.8	5.2	5.6	6.2
5.	Kinetin	10	2.5	4.1	5.0	6.1	7.3
6.	CONTROL	10	9.5	14.0	14.5	15.2	16.0

TABLE XXVI

EFFECT OF PLANT GROWTH REGULATORS ON THE PRODUCTION OF P.M.T.E.

BY P.aphanidermatum

S.NO.	PLANT GROWTH REGULATORS	CONCENTRATION ppm.	% LOSS IN VISCOCITY AFTER MINUTES				
			5	15	30	60	90
1.	Indoleacetic acid	10	3.1	4.4	6.2	8.2	11.6
2.	Indole Butyric acid	10	1.4	1.4	1.7	1.9	2.2
3.	Indole Propionic acid	10	1.3	1.6	2.2	3.1	3.7
4.	Gibberellic acid	10	1.9	2.2	2.2	2.7	3.1
5.	Kinetin	10	3.7	4.3	5.7	6.2	7.8
6.	CONTROL	10	4.0	6.1	8.5	9.1	14.8

TABLE XXVII

EFFECT OF DIFFERENT FUNGICIDES ON THE PRODUCTION OF POLYGALACTONASE

BY P. aphanidermatum

SNO	FUNGICIDES	% LOSS IN VISCOCITY AFTER MINUTES				
		5	15	30	60	90
1.	THIRAM	1.2	1.9	2.1	2.5	3.3
2.	BRASSICOL	5.1	5.7	9.2	10.1	12.3
3.	BLITANE	3.3	5.1	7.3	8.2	10.4
4.	CAPTANE	2.1	3.7	5.2	5.3	6.2
5.	CONTROL	16.0	18.0	28.1	30.0	37.2

TABLE XXVIII

EFFECT OF DIFFERENT FUNGICIDES ON THE PRODUCTION OF PMG BY

P.aphanidermatum

SNO	FUNGICIDES	% LOSS IN VISCOCITY AFTER MINUTES				
		5	15	30	60	90
1.	THIRAM	1.3	2.1	2.9	3.8	4.1
2.	BRASSICOL	2.2	2.3	4.1	5.6	6.2
3.	BLITANE	1.7	2.4	3.6	4.9	5.8
4.	CAPTANE	1.5	2.3	3.1	4.1	5.0
5.	CONTROL	4.0	6.9	10.1	11.3	15.0

TABLE XXIX

EFFECT OF DIFFERENT FUNGICIDES ON THE PRODUCTION OF POLYGALACTRONASE TRANSELIMINASE BY P.aphanidermatum

SNO	FUNGICIDES	% LOSS IN VISCOCITY AFTER MINUTES 5	15	30	60	90
1.	THIRAM	--	1.2	1.7	1.9	2.3
2.	BRASSICOL	5.2	7.1	7.3	7.9	8.1
3.	BLITANE	4.8	6.7	7.0	7.5	7.6
4.	CAPTANE	2.1	3.7	4.2	4.8	5.1
5.	CONTROL	9.5	14.0	14.5	15.2	16.0

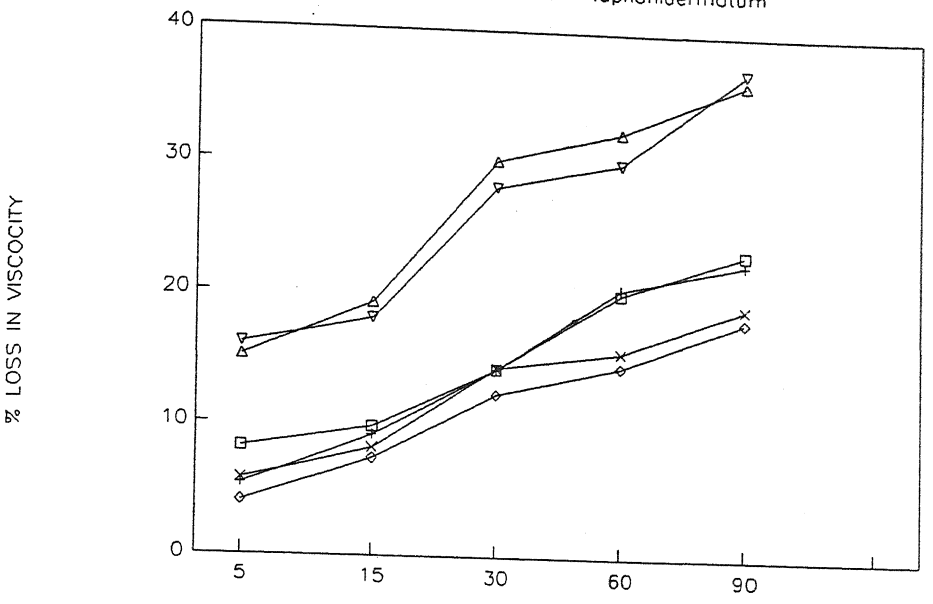
TABLE XXX

EFFECT OF DIFFERENT FUNGICIDES ON THE PRODUCTION OF PMTE BY P.aphanidermatum

SNO	FUNGICIDES	% LOSS IN VISCOCITY IN MINUTES				
		5	15	30	60	90
1.	THIRAM	---	---	---	---	---
2.	BRASSICOL	1.8	2.3	3.7	4.8	6.1
3.	BLITANE	---	---	1.1	1.3	1.4
4.	CAPTANE	---	---	---	---	---
5.	CONTROL	4.0	6.1	8.5	9.1	14.8

EFFECT OF PLANT GROWTH REGULATORS

PG PRODUCTION BY P.aphanidermatum



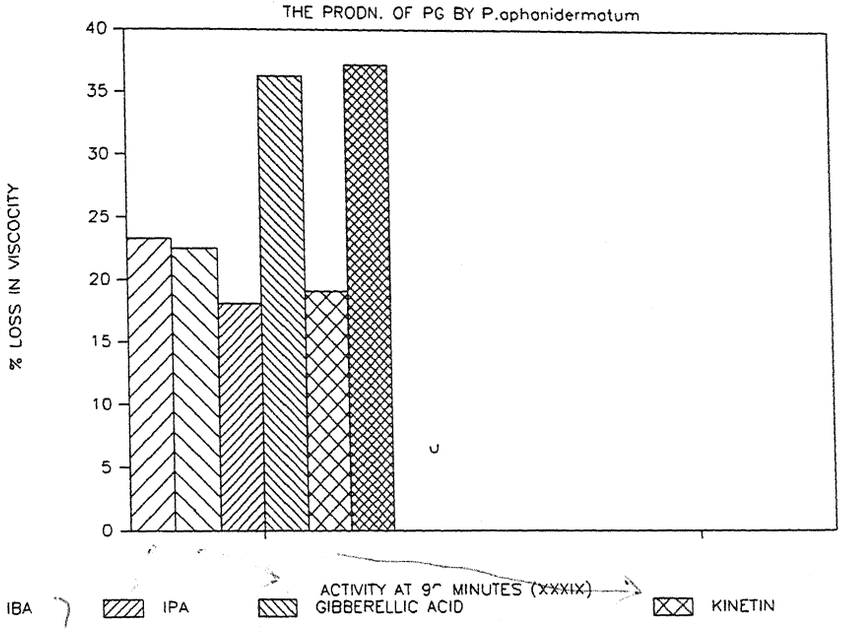
(Fig.XXXVIII)

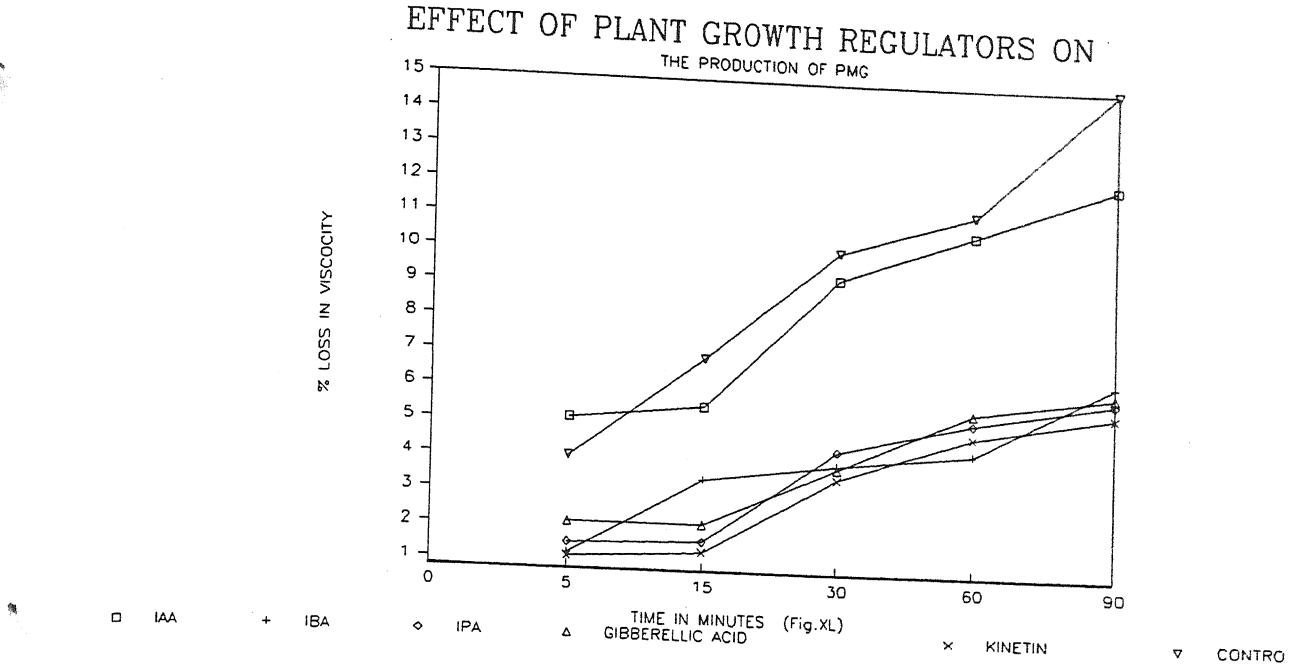
□ IAA + IBA ◇ IPA △ GIBBERELLIC ACID × KINETIN

▽ CONTROL

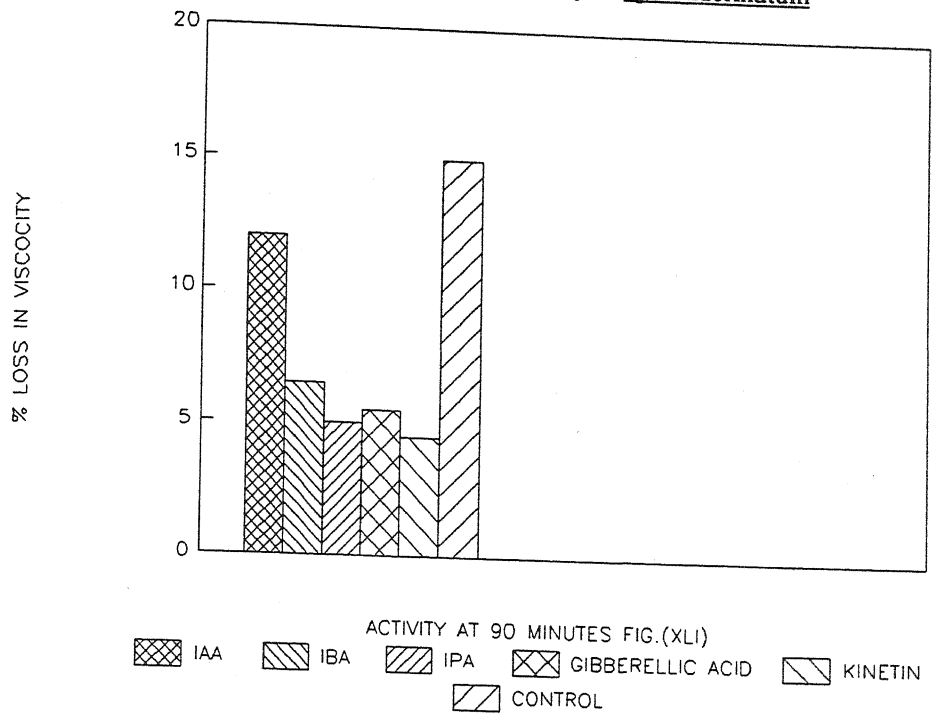
EFFECT OF PLANT GROWTH REGULATORS ON

THE PRODN. OF PG BY P.aphanidermatum



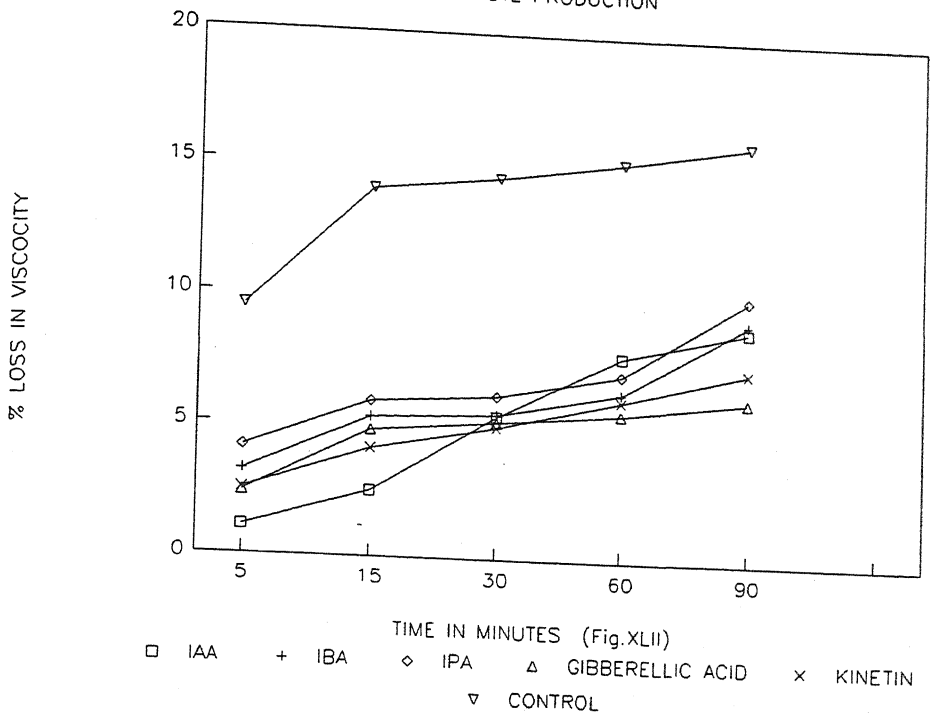

EFFECT OF PLANT GROWTH REGULATORS ON
THE PRODUCTION OF PMG
% LOSS IN VISCOCITY
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
5
15
30
60
90
TIME IN MINUTES (Fig.XL)
GIBBERELLIC ACID
□ IAA
+ IBA
◇ IPA
△
× KINETIN
▽ CONTRO

EFFECT OF GROWTH REGULATORS
ON THE PRODUCTION OF PMG by P. aphanidermatum

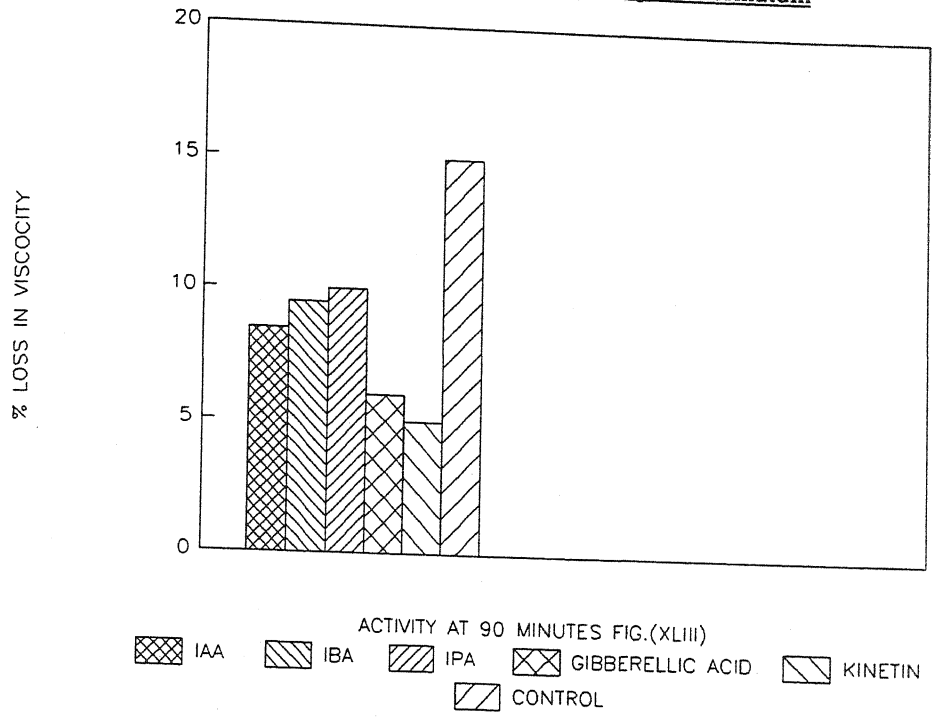


EFFECT OF PLANT GROWTH REGULATORS

PGTE PRODUCTION

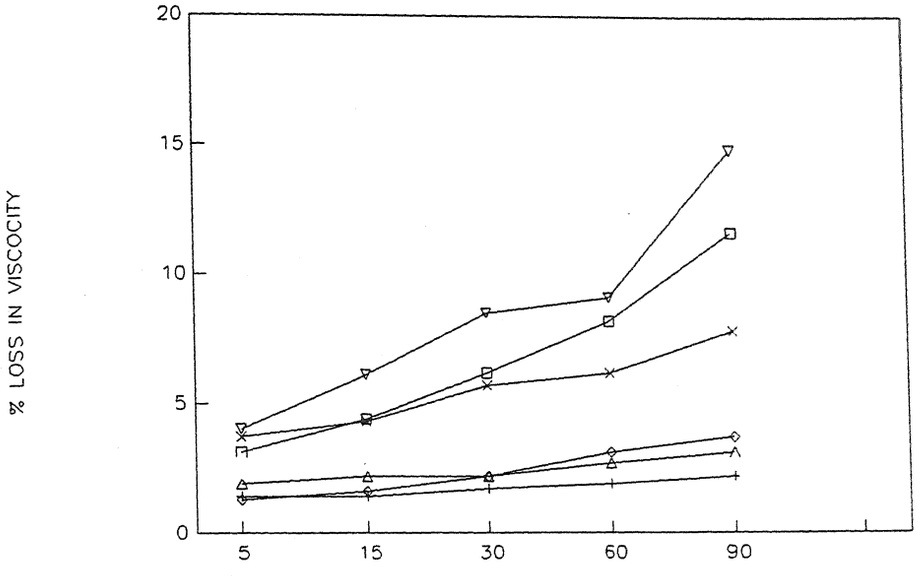


EFFECT OF GROWTH REGULATORS
ON THE PRODUCTION OF PGTE by P. aphanidermatum

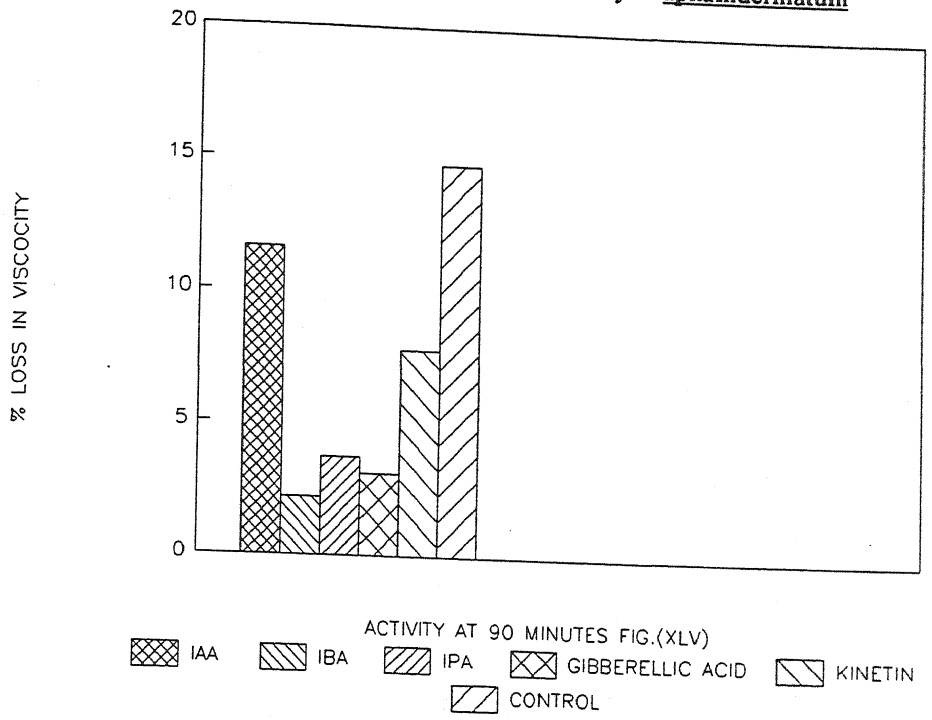


EFFECT OF PLANT GROWTH REGULATORS

PMTE PRODUCTION

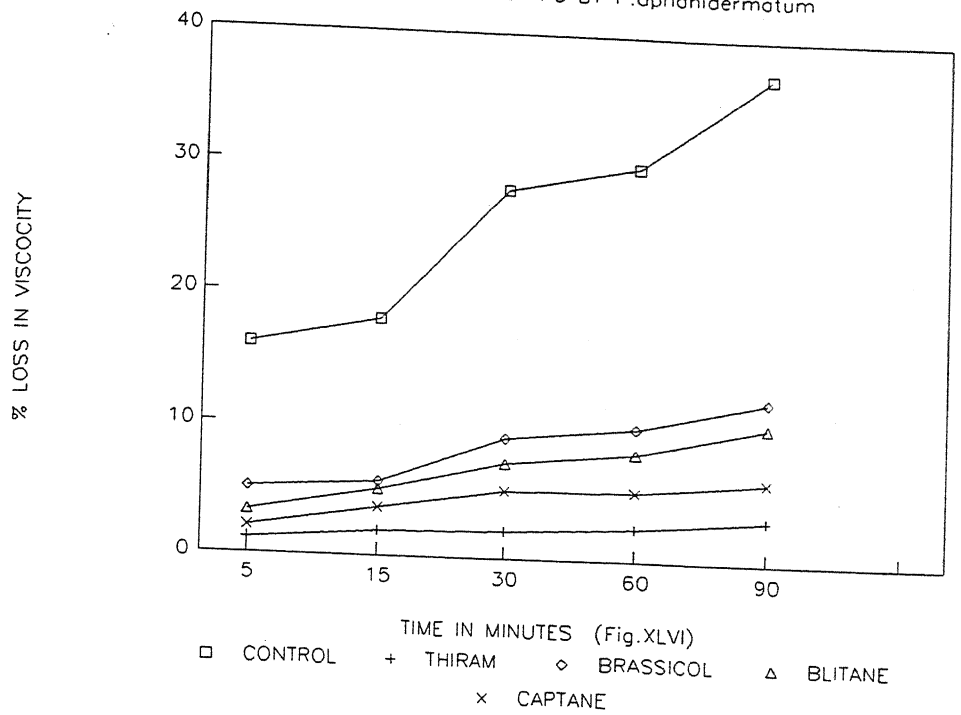


EFFECT OF GROWTH REGULATORS
ON THE PRODUCTION OF PMTE by P. aphanidermatum



EFFECT OF DIFFERENT FUNGICIDES ON THE

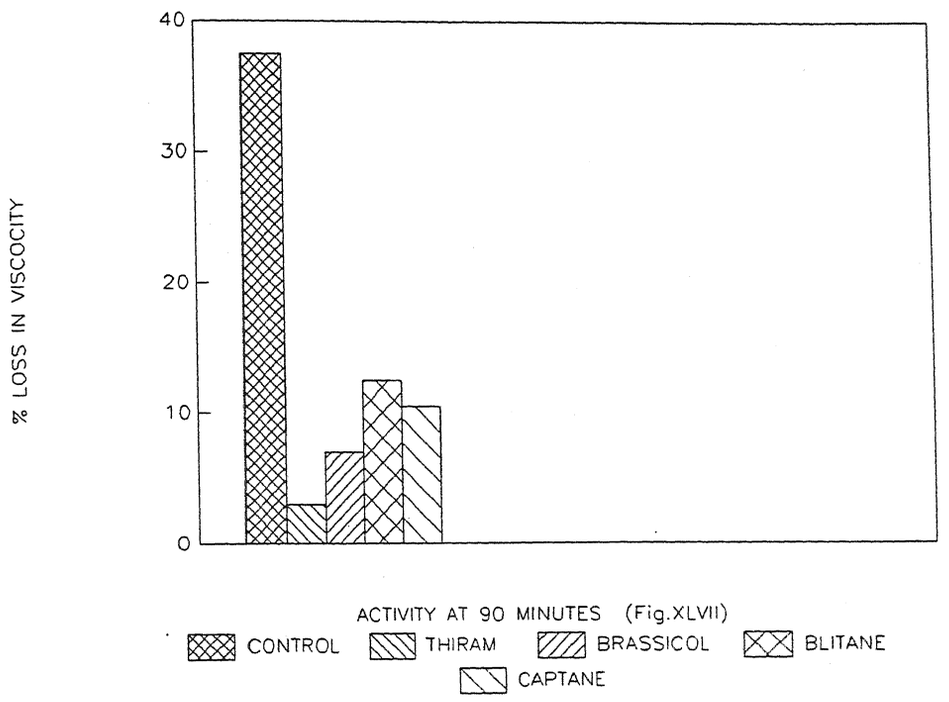
PRODUCTION OF PG BY P.aphanidermatum



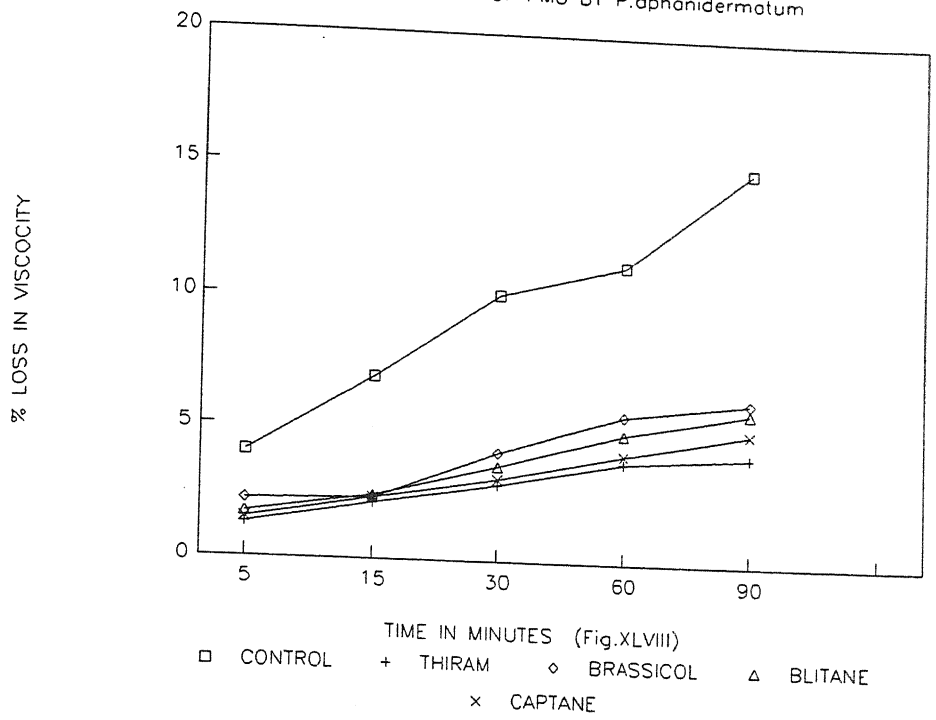
(Fig.XLVI)

EFFECT OF DIFFERENT FUNGICIDES ON THE

PRODUCTION OF PG BY P.aphanidermatum



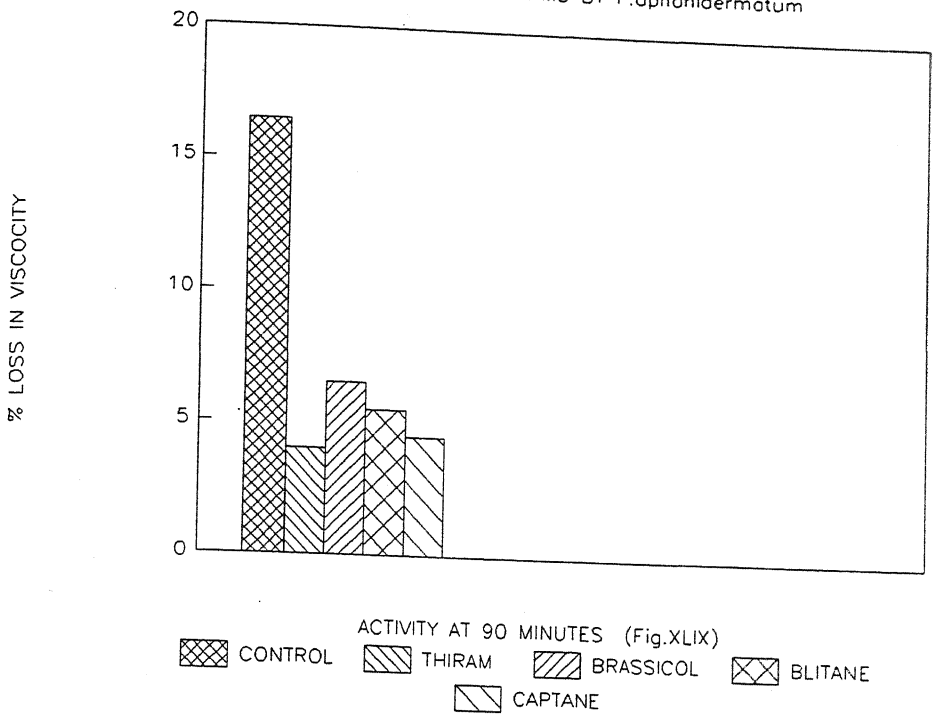
EFFECT OF DIFFERENT FUNGICIDES ON THE PRODUCTION OF PMG BY P.aphanidermatum



(Fig.XLVIII)

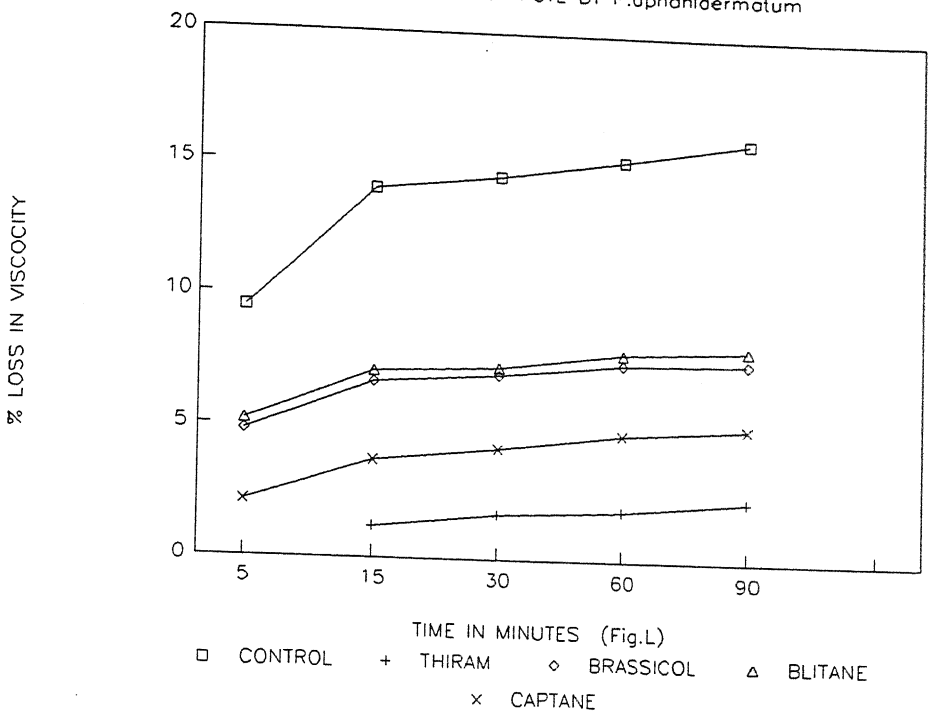
EFFECT OF DIFFERENT FUNGICIDES ON THE

PRODUCTION OF PMG BY P.aphanidermatum



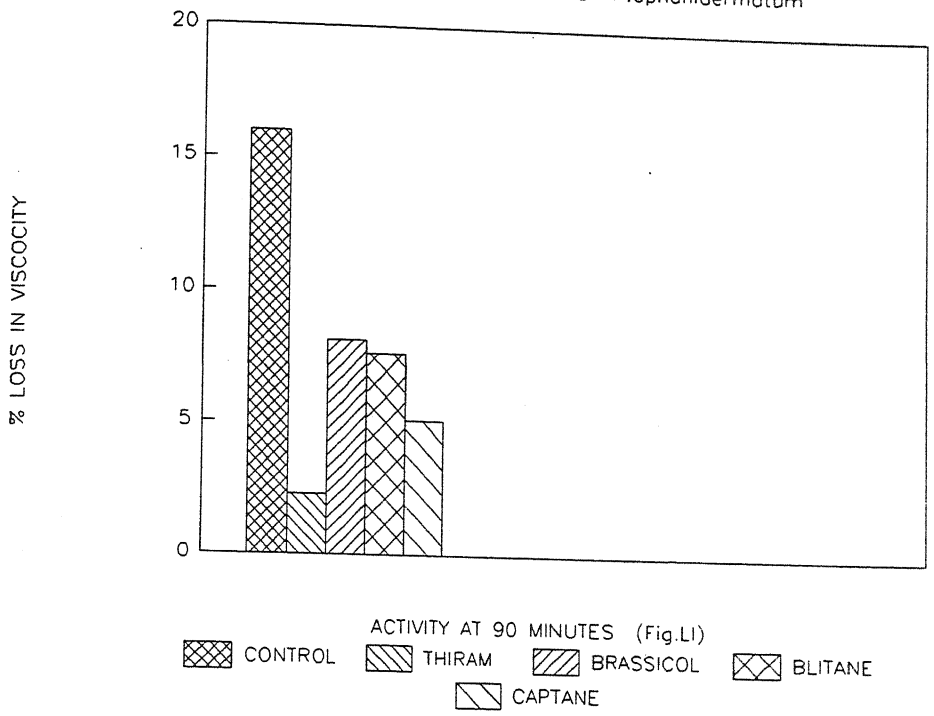
EFFECT OF DIFFERENT FUNGICIDES ON THE

PRODUCTION OF PGTE BY P.aphanidermatum

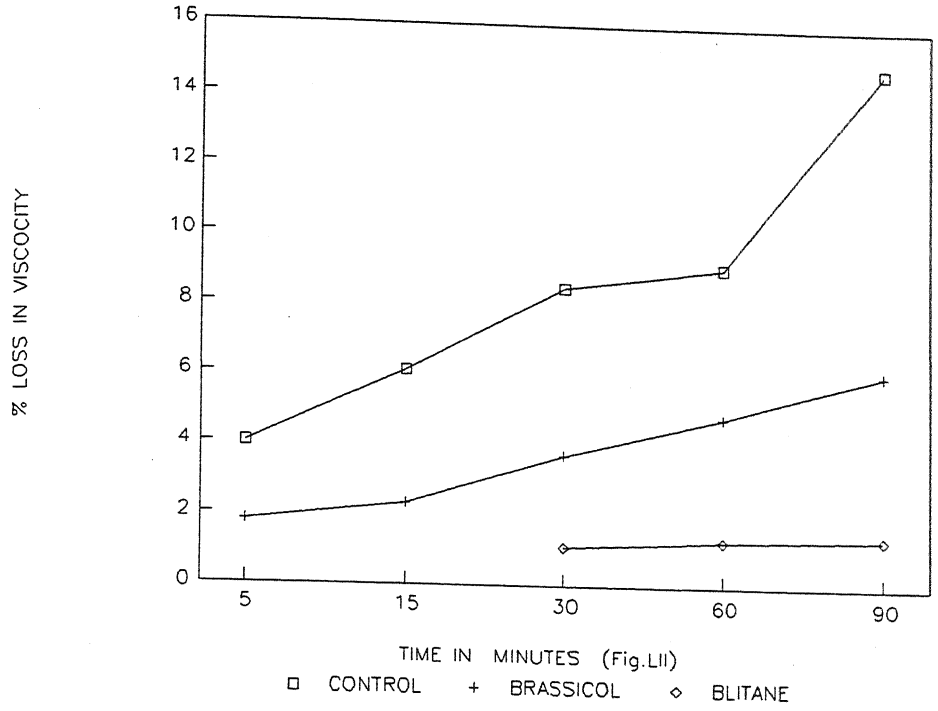


EFFECT OF DIFFERENT FUNGICIDES ON THE

PRODUCTION OF PGTE BY P.aphanidermatum

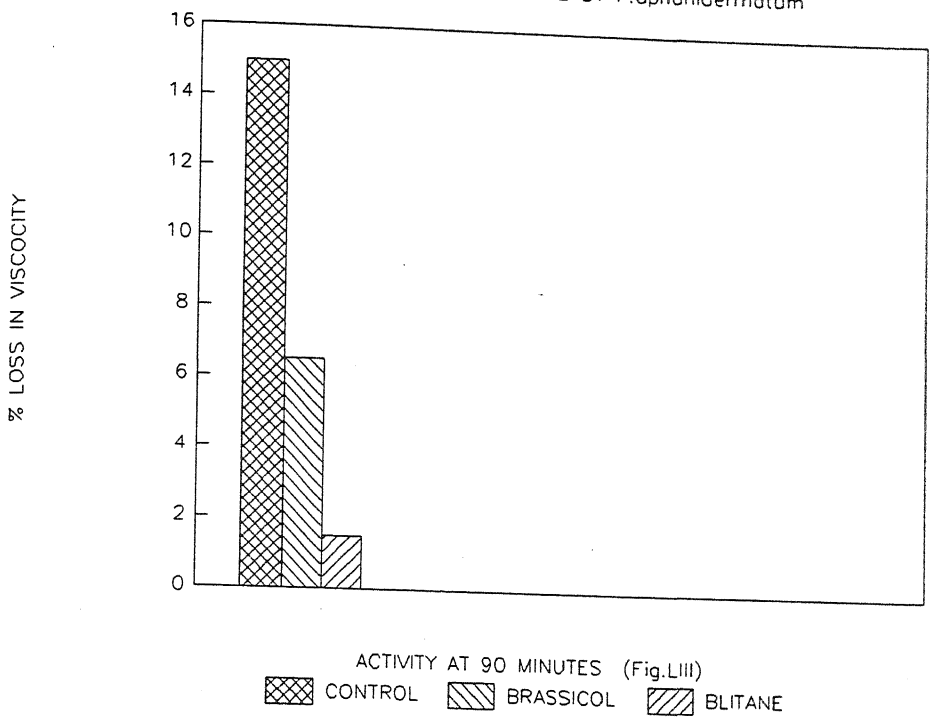


EFFECT OF DIFFERENT FUNGICIDES ON PMTE PRODUCTION by P. aphanidermatum



EFFECT OF DIFFERENT FUNGICIDES ON THE

PRODUCTION OF PMTE BY P.aphanidermatum



CHAPTER XXIII

DISCUSSION AND CONCLUSION

Among the organic substances tested for the inhibitory effects on the production of Pectolytic enzyme were the plant growth regulators and the fungicides. The fungicides were added before sterilization of the Glucose Asparagin medium. While the growth regulators were added after sterilization. and before incubation of the Pathogen. The datas obtained during the studies on various Pectic Enzymes are given in the Table XXIII - XXX1 and Fig XXXVIII to LIII .The results are being discussed below:

PLANT GROWTH REGULATORS

The secretion of Pectic enzymes in culture is significantly affected by all the five plant growth regulators tested .All the hormones caused varying degree of inhibitory effect on various Pectic

Enzymes as mentioned in the previous chapters.In general the enzymes catalyzing the breakdown of methylated chains (PMG & PMTE)were more adversely affected than those responsible for splitting of non-methylated chains of Peptic substances.(PG & PGTE).The synthesis of PG was strongly suppressed by IPA and Kinetin. The decrease in production of PG in the presence of Kinetin have also been reported by Patel and Dimond (1968).The formation of PMG was more adversely affected by Kinetin and IPA.Indol Acetic acid was found to be least effective in reducing the PMG synthesis. With regard to trans eliminases it was observed that inthe case of PGTE again GibberelicAcid and Kinetin caused greater inhibition of enzyme synthesis.PMTE synthesis was more strongly suppressed by IBA followed by Gibberelic acid IAA here again was least effective . However, from the mycelial growth observed andthe secretion of various pectolytic enzymes was observed.On the basis ofover all results Kinetin and Gibberelic acid were found to be the most effective than the others in reducing the pectic enzyme production. It may be recalled that Bateman(1966) has attributed plant disease control or resistance to some of these hormones.

Though the mechanisms involved are not clearly understood but on the basis of the above results it could be suggested that these growth regulators might act as effective agents to control pathogenesis, due to their ability to prevent the synthesis of the pectic enzymes involved during such pathogenesis.

FUNGICIDES

Fungicides in the present study were taken in a concentration of .5% and during

enzyme assay showed very strong suppressive action on pectic enzyme produced by P.aphanidermatum . Thiram , Brassicol, Blitane and Captane were the fungicides used in the present study . The formation of Poly Galactronase enzyme by P. aphanidermatum was strongly suppressed by the Thiram and Captane. Table XXVII & Fig. XLVI & XLVII.These results are similar to those observed by Rai(1971) . PMG production was also considerably reduced by Thiram and Captane. Table XXVIII & Fig. XLVIII , XLIX. PGTE was also inhibited significantly by Thiram.Table XXIX, & Fig. L & LI. On PMTE both Thiram and Captane were so adversely affected that no enzyme production could be noticed. Table XXX & Fig. LII & LIII. The over all result show that all the fungicides were very significantly effective in reducing the pectic enzyme production . Thiram and Captane weremost effective in reducing the enzyme production .

The inhibition of pectic enzyme production by various fungicides on several fungal pathogens have also been reported by Grover and Moore (1962) Grover (1964).

PART II
SECTION 'B'

CHAPTER XXIV

INTRODUCTION

Cellulose forms an integral part of plant cell walls & is the most prevalent organic constituent.It is composed of long chain of glucose molecules linked through C1,4 by B-glycosidic linkages.Chain length may be 1-3 with a degree of polymerization of 2000 to 10,000 units.Cellulose is organised in the cell walls as the parallel cellulose chains forming microfibrils. These microfibrils are the basic structural units of the cell wall. These are non amorphous components of the cell wall & it has been sucessfully demonstrated that they form the skeleton of an expanded cell wall. Removal of non-cellulosic constituents of such walls involve little change in cell form & does not alter the mechanical property of the cell wall. .The space between the microfibrils are filled with pectins as the primary encrusting substance.

Native cellulose is insoluble but water soluble products are formed when primary & secondary hydroxyl groups of glucose units [illegible] are replaced by certain other groups such as methyl and carboxymethyl groups.

The following cellulolytic enzymes are known to be involved during the degradation of native cellulose.

(a) Enzyme C_1 which acts on native cellulose liberating long glucose anhydride chains. This enzyme cannot degrade cellulose to the lower level.

(b) Enzymes Cx which acts on the substrate liberated by C1 enzyme and brings it to disaccharide or hexose level.

B-glucosidase, also known as Oligo-B-glycosidase or cellobiase may be involved in splitting of the soluble products prior to cell wall metabolism, Norkans (1963), Wood(1967), proposed a scheme of cellulolytic enzymes when native cellulose is degraded.

Native Cellulose

|

Enzyme C_1

Linear Chain — Modified Cellulose

Enzyme C_x — Enzyme C_x

Cellobiase

|

C_x or B-glucose

Glucose

Glucose is the end product after complete enzymatic degradation of cellulose . It is produced by the action of C_x enzyme on the products liberated by the action of C_1 enzyme on native celulose. It is agreed by some workers that C_x produces cellobiose which is degraded to glucose by the action of cellobiose or B-glucosidases. Recently it has been suggested that the group of enzymes referred to as C_x may be regarded as B Glucoses.1,4.

These enzymes are liberated by all the succesful pathogens which attack plant tissues. During the pathogenesis of the rhizome rot of ginger these enzymes must be playing an important role. Thus the study of cellulolytic enzyme was considered to be important in understanding plant disease syndrome. With the above considerations the study in this section was planned under the following lines.

Subsection (a):Invivo studies of cellulolytic enzymes:

Under this subsection the presence & activity of cellulolytic enzyme was studied both in healthy rhizomes & in the diseased rhizomes inoculated with Pythium aphanidermatum

Subsection (b)Invitro studies of cellulolytic enzyme under various cultural conditions:

Under the subsection the capacity of cellulolytic enzyme production by Pythium aphanidermatum was studied under various cultural condition i.e in different media, different incubation period and the effect of pH.

Subsection (c):In vitro studies of cellulolytic enzymes in presence of growth regulators and fungicidesUnder this subsection the effect of various plant growth regulators & fungicide were studied on the cellulolytic enzyme produced by Pythium aphanidermatum.

CHAPTER - XXV

REVIEW OF LITERATURE

The degradation of cellulose has been reviewed in detail by Norkans (1963-b). He has also described the influence of cultural condition on fungal cellulose production (1963-a).

Chemically cellulose is high polymer of β likage of $D-_{1,4}$ glucose residues. The chain molecule of the native cellulose are of considerable length varying with different species & different tissues.Cellulosic substances are aggregate of glucose an-hydride chains arranged more or less parallel to each other. It is often regarded as a skeletal substance irreversibly deposited in plant cells & not broken down by enzymes of endogenous origin. This is true for mature plants tissues. In young cells however breakdown & resynthesis of cellulose in the primary wall has been found. Degradation of such material is neccessarily involved in the process of germination. It is also broken down through the agency of bacteria & fungi representing differential taxonomic group.Some bacteria & fungi even Phycomycetes causing common diseases such as soft rot (Winstead & McCoumbs (1961)). Cellulolytic enzymes seems to be a prerequisit. Rees et.al. (1953) have presented extensive list of genera & species involved in this determination.

Cellulose are generally considered to be formed only in the presence of cellulase. According to Reese & Mandle (1963) not cellulose itself but the soluble cellulose are hydrolysis product, in this line inducer of cellulose in cellulose culture but when cellobiose is used in amount as large as those in regular growth experiments always depressed the cellulase yield and was also the case in glucose together with cellulose was used as the carbon source. Husain & Diamond (1960). In some cases cellulase is produced even in the absence of cellulose Lyr et.al.(1959), Reese (1963) calls attention to the fact that nutrient solutions contains supplimentary additions of 12% Malt extract which might be responsible for cellulose induction. There seems to be no Thermodynamic or other fundamental reason for supposing that cellulose symptoms in all organism must neccesarily be induced.When testing with 124 fungi for the production of the cellulolytic enzyme Basu & Ghosh (1960) found that only limited number of fungi produce celluloses when grown on pure cellulose but most of the organism produce cellulose when grown on mixed cellulosic substrate. The mixed substrate, have organic & inorganic micro nutrients that facilitate the enzyme secretion. Yeast extract for instance has been shown to produce cellulose production. Norkans & Ascham (1953).

In culture filtrates of fungi extra cellulase can be demonstrated. These are released from viable cells Mandel (1956). During the later phase increased cellulase activity can be observed,

presumably not due to release of cellulase, this may be due to the release active proteins by autolytic process from the surface and from internal part of the cell.

Norkans (1963-a). The importance of cultural conditions for cellulose production have been pointed out by Whitaker & Thomas (1963). The Oxygen supply obtained and the relation of culture liquid, volume of culture vessel play an important role. Mechanical shaking or bubbling has a deleterious effect on enzyme produced. Norkans (1963-a).

There are two different enzymes involved in the breakdown of mature cellulose it is said to produce an hydro glucose chain from mature cellulose. Subsequently hydrolysis the chain to soluble low molecular product ranging from cello hydrose to glucose. The process can be expressed as follows.

$$\text{Native cellulose} \xrightarrow{C_1} \text{Linear anhydroglucose Chains} \xrightarrow{C_x} \text{Soluble low molecular products.}$$

Cx enzyme were also found by Winstead & McComb (1961) in culture filtrates and cucumber fruits extract. Here cellobiose was degraded to glucose. Cx activity of culture filtrate from 14 days old cultures was much greater than 3 & 7 days old cultures.

Cellulolytic enzymes have been found to play a significant role in some plant disease. Bateman (1969) carried out a detailed investigation of the enzyme on S.rolfsii & demostrated that the culture filtrates & water extracts of lesions induced by this pathogen on bean hypocotyls contained system (C_x type) that exhibit optimum activity at 4pH on carboxyl methyl cellulose (CMC).

Norkans (1963-b),suggested that cellulolytic enzyme were pre-requisite in certain wilt disease. Bateman & Millar (1966) are of he view that cellulose does not play any significant role in the maceration of plant tissue. According to Shrivastava et al.(1959), Bateman (1964) cellulose has been obtained from diseased tissue,Wood(1960) suggested that cellulose do appear to be very important in early stages of disease development but in the later stages of soft rot cellulose of the microfibrils is definitely attacked by soft rot pathogens.

Brown (1965) reported the S. rolfsii produced cellulase in the presence of an inducing substrate in culture & indicated its probable role in pathogenesis.

Cellulase deterioration by fungi has been determined extensively by Great House *et.al*. (1942). Cellulose & ligno cellulose degradation by thermophillic & thermotolerent fungi has been worked by Rosenberg (1978). Microbial decomposition of cellulose has been reviewed by Sin (1951). The invitro studies of cellulolytic microflora has been worked by Bose &

Yadav (1973). Bisht & Harsh (1981) has even observed the envolvement of wood decaying fungi on cellulose. Review on various Pectolytic & Cellulolytic enzyme involvement in tissue degradation & maceration has been given by Mehrotra & Goel (1978).

Effect of fungicides including antibiotic on cellulolytic enzyme activity of Rhizoctonia bataticola has been described by Goel & Mehrotra (1973,74).

Comparitively little work has been done in India regarding cellulolytic enzymes especially with reference to their role in pathogenesis.

PART II

SECTION 'B'

SUB SECTION 'a'

CHAPTER XXVI

INTRODUCTION

INVIVO STUDIES OF CELLULOLYTIC ENZYME

Cellulose is the most prevalent organic substance present in the cell wall of plant tissues. In pathogenesis cellulose degrading enzymes are comparatively less worked out because Plant Plathologist have generally paid more attention to the Pectolytic enzymes during pathogenesis.Work of Bateman (1969), Hancock & Millar (1965), Husain & Diamond (1960),Rai (1971), Vyas (1971) and many others have proved that the role of cellulose degrading enzyme during pathogenesis is beyond doubt. In the present section therefore the production & activity of cellulolytic enzyme in vivo & in vitro have to be studied in the following lines.

(i) Cellulolytic enzymes in the Healthy Ginger Rhizomes.

(ii) Cellulolytic enzymes in the diseased Ginger rhizomes infected with Pythium aphanidermatum.

CHAPTER XXVII

EXPERIMENTAL

The method for extractions of enzymes from healthy & diseased ginger rhizomes were the same as used for the extraction of pectic enzymes in the previous chapter. The method of inoculation & incubation were also the same.

METHOD FOR ENZYME ASSAY

Standard Viscometric procedure was use d for analysing the cellulase ($C_x$type) activity. In this case the substrate X was 1.2% solution of carboxymethyl cellulose (CMC). The enzyme activity was measured for reducing the viscosity of CMC and was the same as described for pectic enzymes. The enzyme reaction mixture used was as follows —

1.2% Carboxy Methyl Cellulose	—	3.5 ml
Distilled Wate	—	1.5 ml
Mcallvain Buffer (pH 5.5)	—	1.5 ml
Enzyme Extrac	—	1.5 ml

For control enzyme reaction mixture with autoclaved enzyme extract was run simultaneously.

Enzyme activity was expressed as percentage loss in viscosity. The relative enzyme activity was determined according to the formula given earlier.

CHAPTER XXVIII

RESULTS AND OBSERVATIONS

CELLULOLYTIC ENZYMES IN HEALTHY & DISEASED RHIZOMES

Extracts of healthy & diseased rhizomes when assayed for cellulolytic enzyme activity showed a less significant C_x type of activity. The diseased rhizome after 5 days of incubation gave almost the same activity as that observed in healthy rhizomes, though there was a slight increase in the diseased rhizome but it is almost insignificant. As the incubation period was increased to 10th day, the C_x activity was slightly enhanced. It could be said that as the incubation period increased the enzyme activity increased and 10 days incubation period gave better cellulase enzyme production as compared to those of 5 days of incubation period.Table XXXI & Fig. LIV,LV.

TABLE XXXI

CELLULOSE ACTIVITY IN GINGER RHIZOME INFESTED WITH P. aphanidermatum

Days of incubation	% Loss in viscocity after minutes					R.E.A.
	5	15	30	60	90	
0	6.4	8.2	9.1	11.0	12.5	-
5	6.5	9.2	11.2	12.3	13.4	-
10	7.5	9.4	14.2	16.1	17.8	-

REA AT 25 % LOSS IN VISCOCITY

CELLULASE ACTIVITY IN HEALTHY AND IN

P.aphanidermatum infected rhizomes

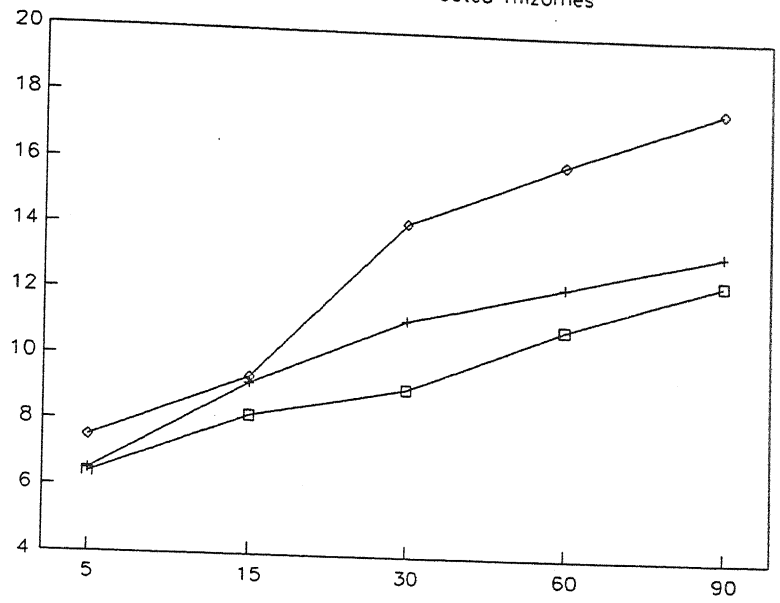
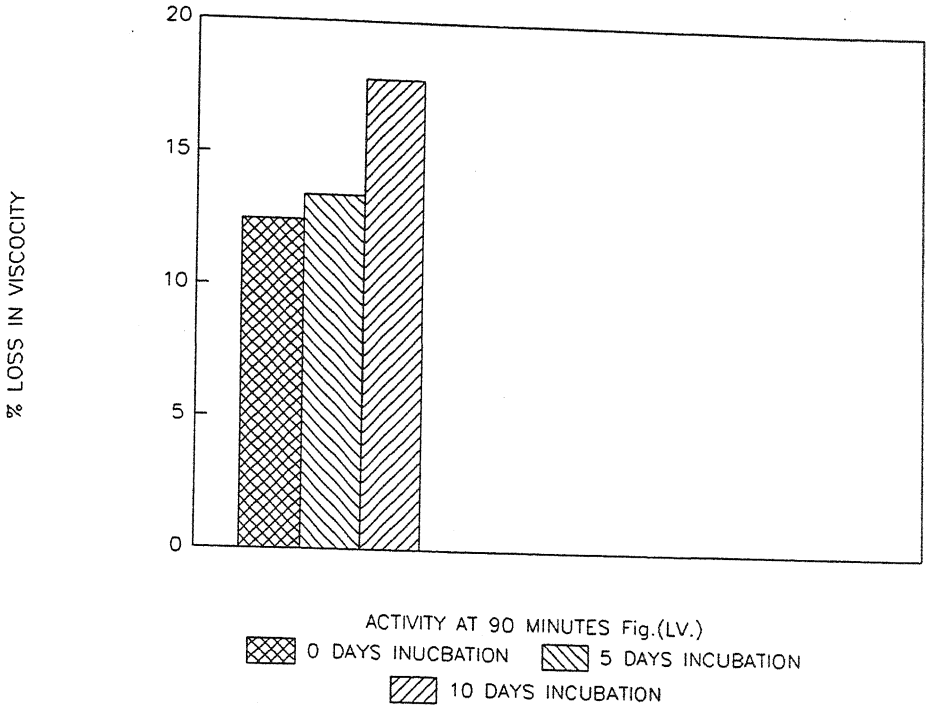


Fig.(LIV.)

□ 0 DAYS INCUBATION + 5 DAYS INCUBATION

◇ 10 DAYS INCUBATION

CELLULASE ACTIVITY IN HEALTHY AND IN INFECTED RHIZOMES



PART II

SECTION 'B'

SUB SECTION 'b'

CHAPTER XXIX

Discussion & Conclusion

The extract of healthy & diseased rhizhome infected with Pythium aphanidermatum showed C_x type of cellulase activity. The results obtained in Table XXXI & Fig LIV,LV show that the activity in the case of diseased rhizome was only slightly higher than that of fresh rhizomes after 5 days of infection. As the incubation or infection period was increased to 10 days cellulose activity increased this indicates that cellulose enzymes are involved in pathogenesis during development of soft rot of ginger by Pythium aphanidermatum. Similar involvement of cellulase enzymes during pathogenesis have been observed by number of workers.Bateman (1964) and others. The presence of cellulase activity in healthy tissue of ginger rhizome have been observed by the present worker:. The observation is in line of the observations of Bateman (1964), & Rai (1971).

CHAPTER XXX

INTRODUCTION

IN VITRO STUDIES OF CELLULOLYTIC ENZYMES UNDER VARIOUS CULTURAL CONDITIONS

The presence of the cellulolytic activity during pathogenisis of P. aphanidermatum provides convincing reasons for studying the enzyme activities in vitro. The primary object of this study is to find out the effect of various culture media, incubation period and pH on the production of cellulose enzyme production by the P. aphanidermatum. The experimental work has been planned in the following lines.

(i) Effect of different culture media on the production of the cellulolytic enzyme by P. aphanidermatum

(ii) Effect of pH on the production of cellulolytic enzymes by P. aphanidermatum.

CHAPTER XXXI

EXPERIMENTAL

P. aphanidermatum was the one obtained from the diseased rhizomes in the previous section.

Effect of culture media:

The different cultural media used here were the same used during the study of pectic enzyme i.e.,

1. Potato Dextrose
2. Czapecks Dox
3. Peptone Dextrose
4. Glucose Asparagine.

The incubation period is also the same i.e.,5 days and 10 days.

Effect of pH

Here also the pH range selected for the study was the same i.e., from 2.5-12. The method for adjusting pH was the same as described previously in case of pectic enzymes. The only difference was that here studies for pH were conducted on 10 days old culture.

The exhibition of enzymes and the method of enzyme assay were the same as described in the earlier chapter for cellulase activity.

CHAPTER XXXII

RESULTS AND OBSERVATIONS

1. Effects of different culture medias on the production of cellulose by P. aphanidermatum

It is clear from the datas given in the table XXXII Fig. LVI - LIX that in general,cellulase production was variously favoured by different media. However the amount of activity observed in each case was found to vary in different incubation period and different culture media. Glucose Asparagin was most favourable for cellulase production by P. aphanidermatum. Peptone Dextrose and Czapecks Dox was less effected while Potato Dextrose was least favourable. Cellulase production was also influenced by period of incubation. It was observed that when the incubation period was increased from 5 to 10 days the enzyme production increased in Glucose Asparagin as well as Potato Dextrose. But in Czapecks Dox and Peptone Dextrose the enzymes activity was almost the same and was not much effected by increasing the incubation period.

2. **Effect of pH on the production of cellulose by P. aphanidermatum**

It is evident from the result shown in the table XXXIII Fig.LX - LXI that for the secretion of cellulase,the acidic range of pH i.e., 3-5 was most favourable and pH 4 was found to be optimum. No cellulolytic activity was found below pH3 and above pH 8. The cellulase activity progressively decreased from pH 5 to pH 8.

TABLE XXXII

EFFECT OF CULTURE MEDIA ON THE CELLULOSE PRODUCTION BY P.aphanidermatum

		Cellulose Activity After 5days & 10days of Incubation											
		5days %loss in viscocity after min						10days %loss in viscocity after min					
S.n	MEDIA	5	15	30	60	90	REA	5	15	30	60	90	REA
1.	Potato Dektrose	11.1	12.0	15.7	20.1	24.4	-	13.1	15.2	19.8	22.1	29.8	13.93
2.	Czapecks Dox	13.7	15.8	19.6	27.1	34.0	19.37	18.0	20.0	23.4	26.2	33.7	21.50
3.	Peptone Dektrose	14.2	16.3	20.2	28.5	34.8	21.35	15.3	19.7	25.6	30.1	35.5	35.08
4.	Glucose Asparagin	20.1	23.4	27.2	35.1	40.0	45.97	25.1	30.2	35.6	42.2	50.3	200.00

REA AT 25% LOSS ON VISCOCITY.

TABLE XXXIII

EFFECT OF pH ON THE CELLULOSE PRODUCTION BY *P.aphanidermatum*

Initial pH	% Loss In Viscocity After Minutes				
	5	15	30	60	90
2.5	---	---	---	--	---
3	13.1	18.1	25.3	29.3	35.1
4	35.1	40.3	43.8	49.1	57.1
5	19.6	21.7	25.1	32.3	37.7
6	19.1	11.3	16.2	18.1	20.8
7	8.2	10.1	12.0	14.2	16.7
8	3.9	5.4	7.1	8.2	8.5
9	---	---	---	---	---
10	---	---	---	---	---
11	---	---	---	---	---
12	---	---	---	---	---

EFFECT OF DIFFERENT CULTURE MEDIA

ON CELLULASE PROD'N AFTER 5 DAYS INCUB

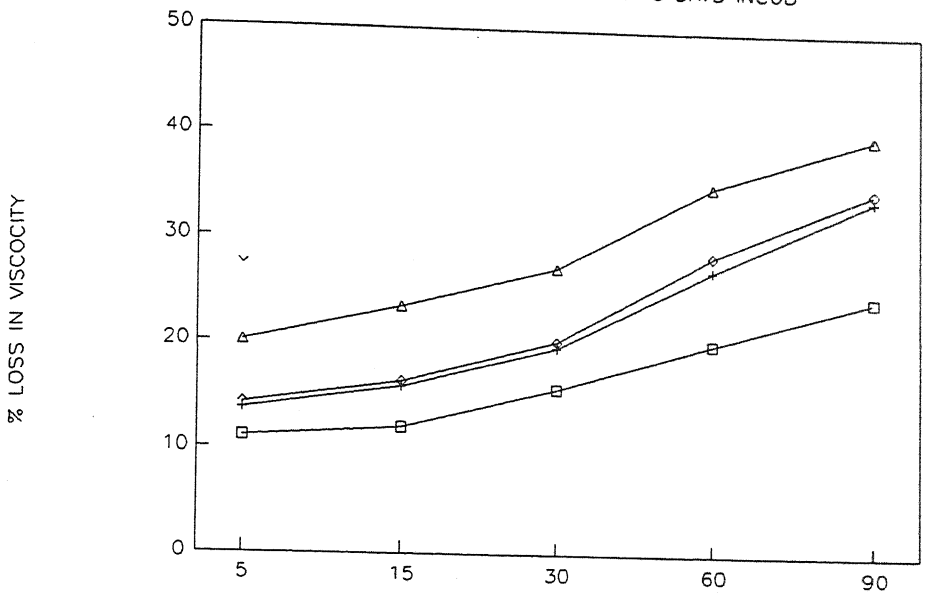
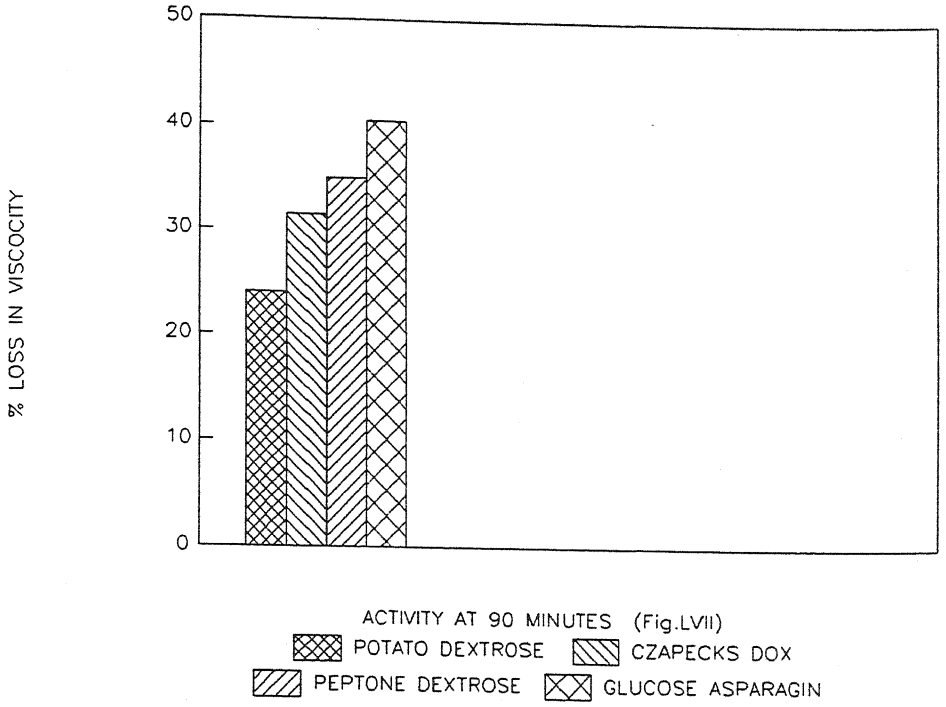


Fig.(LVI.)

□ POTATO DEXTROSE + CZAPECKS DOX

◇ PEPTONE DEXTROSE △ GLUCOSE ASPARAGIN

EFFECT OF CULTURE MEDIA ON CELLULASE PRODUCTION AFTER 5 DAYS INCUBATION by P. aphanidermatum



EFFECT OF DIFFERENT CULTURE MEDIA
ON CELLULASE PRODUCTION AFTER 10 DAYS OF INCUBATION

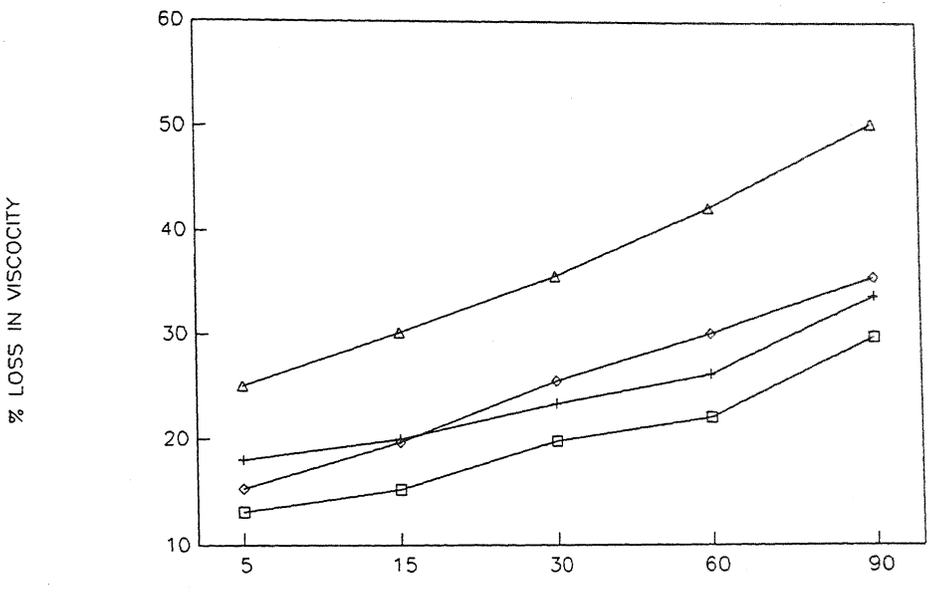
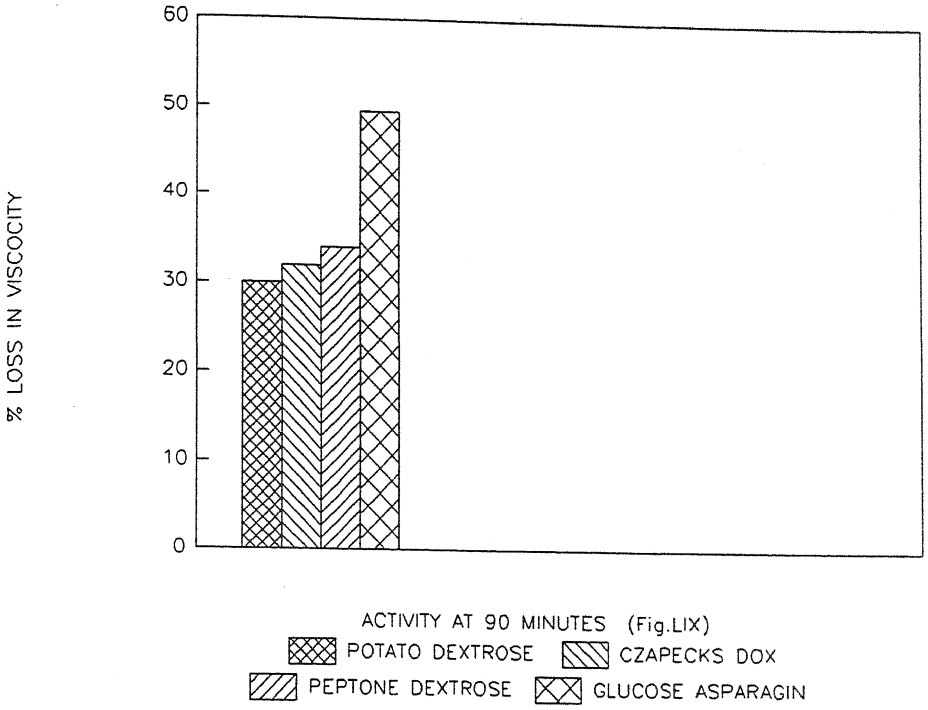


Fig.(LVIII.)

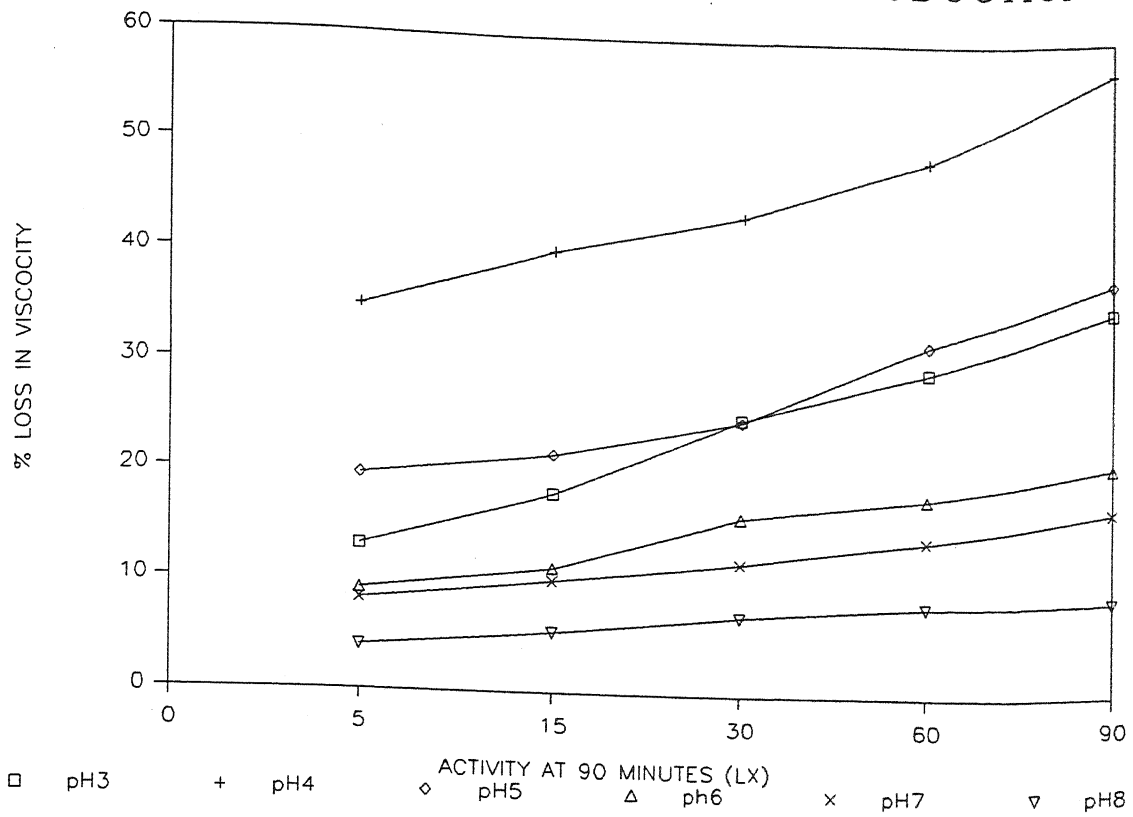
□ POTATO DEXTROSE + CZAPECKS DOX

◇ PEPTONE DEXTROSE △ GLUCOSE ASPARAGIN

EFFECT OF CULTURE MEDIA ON CELLULASE PRODUCTION AFTER 10 DAYS INCUBATION by P. aphanidermatum

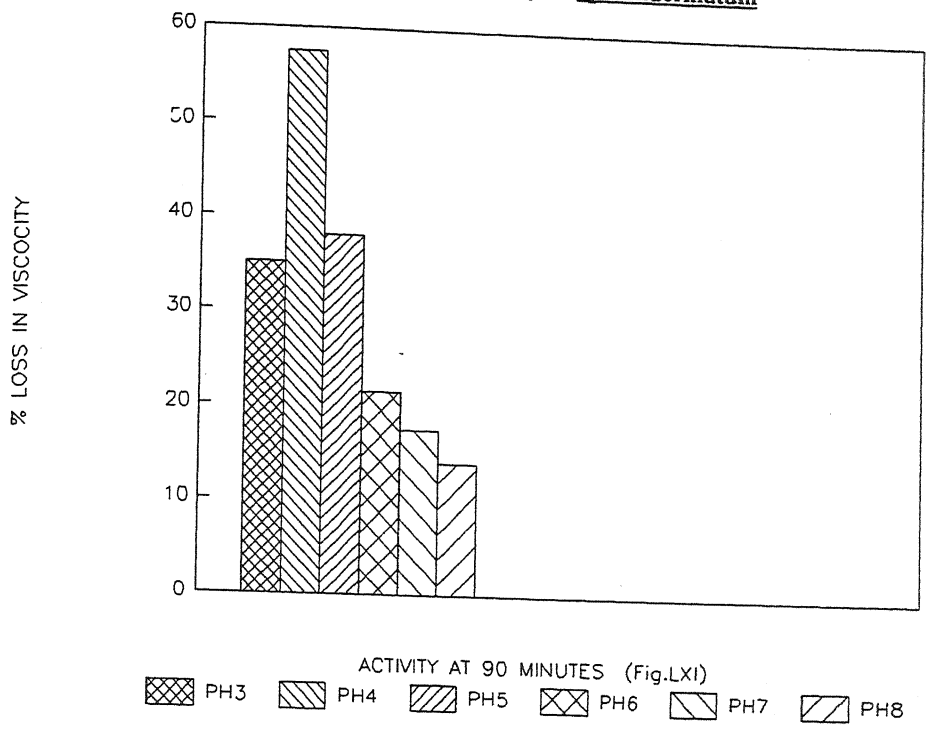


EFFECT OF pH ON CELLULASE PRODUCTION



EFFECT OF pH ON CELLULASE PRODUCTION

PRODUCTION by P. aphanidermatum



PART II

SECTION 'B'

SUBSECTION 'c'

CHAPTER XXXIII

DISCUSSION & CONCLUSION

To determine the optimum cultural conditions for production of cellulolytic enzymes byP. aphanidermatumthe different cultural media,incubation period and the pH was studied. The result obtained in the previous chapter have been discussed below:

1. Culture Media:

Out of the 4 culture media i.e., Potato Dextrose, Czapecks Dox, Peptone Dextrose and Glucose Asparagin. Glucose Asparagin was most favourable for the synthesis of cellulase. Potato Dextrose was the least. Ali (1970) has also found that PDB favour very little cellulase secretion. Rai (1971) has also observed that PDB was least effective for cellulase production. Secretion of enzymes was also affected by different incubation period with different media. The overall study shows that Glucose Asparaginemedium with 10 days incubation period gave good results. Therefore, this media was selected for further study on the effect of pH on cellulase production by P.aphanidermatum.. The observations for the growth of P. aphanidermatum in different media shows that the growth was maximum on PDB and minimum on Glucose Asparagin. These observations suggest that mycelial production has no correlation with cellulase production by P. aphanidermatum.

2. Effect of pH

Maximum amount of cellulase secretion was observed at pH4. This observation of the author is also similar to those of Ali (1970) and Rai (1971). Below pH3 and beyond pH 8 there was no cellulase production by P. aphanidermatum. Lower pH values (3-5) have a higher cellulase production. These results are similar to those of Mandel & Reese (1965) on Trichoderma viride.

CHAPTER XXXIV

INTRODUCTION

IN VITRO STUDIES OF CELLULOLYTIC

IN PRESENCE OF GROWTH REGULATORS AND FUNGICIDES

Cellulase production is increased by certain substances while may be reduced by certain other substances. The substances which reduces cellulolytic activities may be useful from Chemotheraputic point of view while inducers of cellulase may be applied for industrial purposes. A number of workers however observed that Cx type of cellulase may be produced in culture even in the abscence of cellulosic material.Rai (1971),Vyas (1971).

The author is interested in finding out the repressors for cellulase production as such components could act as interesting tool for plant disease control. Some substances have been found to be good for cultural growth or reducer for such growth,but from the point of view of cellulase production these substances are less worked out. The compounds which may act as reducers are growth regulators and fungicides. The inhibitation of cellulase has been reviewed by Mandle & Reese (1965) but in most of these studies work has been carried out by taking culture filtrates of fungal pathogens and then the effect of these organic substances has been investigated by adding these substances in the reaction mixture for enzyme assay. These results are actually the effect on the activity of cellulase rather than the development of cellulolytic enzyme in culture. In view of this in the present study the effect of various plant growth regulators and fungicides has been investigated on the production of cellulase by P. aphanidermatum in culture. The present investigations has been done in the following lines.

1. Effect of plant growth regulators on the production of cellulase enzyme by P. aphanidermatum.

2. Effect of fungicides on the production of cellulase by P. aphanidermatum.

CHAPTER XXXV

EXPERIMENTAL

Glucose Asparagine media was used in this study also as high cellulase activity recorded in this media. The media was incubated for 10 days. Control was maintained in both the experiments in which Glucose Asparagine medium was taken without adding either growth regulators or fungicides. Growth regulators were added after sterilization in 10 ppm concentration and fungicides were added in .5% concentration before sterilization. Procedure for extraction of enzymes and assay of enzymes was the same as used previously for cellulolytic enzymes.

CHAPTER XXXVI

RESULTS AND OBSERVATIONS

GROWTH REGULATORS -

Data shown in the table XXXIV and Fig.LXII - LXIII indicate that all the five growth regulators caused a substantial loss of cellulase production. Among these growth regulators IBA was most effective followed by Gibberellic acid and Kinetin. IAA however showed that the least inhibitory effect. During the course of preparation for enzyme extract the author observed that in broth cultures these growth regulators produced a fair amount of growth of P. aphanidermatum. It appears that the toxic effect of these regulators were less pronounced on the systematical growth as compared to the cellulase production.

FUNGICIDES

Cellulase production by P. aphanidermatum was also studied in the presence of fungicides and the results obtained are given in the table XXXV Fig. LXIV - LXV . From the datas given it can be observed that these fungicides are more effective in decreasing cellulase production by P. aphanidermatum . These fungicides were also very effective in decreasing the fungal growth over the broth culture developed for enzyme extract preparation. Among these fungicides Thiram caused maximum loss in viscocity after substract CMC. Brassicol was least toxic in which the cellulase production was maximum only a very slight mycelial growth was found on the surface of the broth culture in the flask. Next to Thiram was Captane which also remarkably reduced the enzyme production by P. aphanidermatum..

From the over all result it could be said that the fungicides were more effective in reducing mycelial growth as well as enzyme production compared to those of the plant growth regulators.

TABLE XXXIV

EFFECT OF PLANT GROWTH REGULATIONS ON THE PRODUCTION OF CELLULOSE

P.aphanidermatum (AFTER 10DAYS OF INCUBATION)

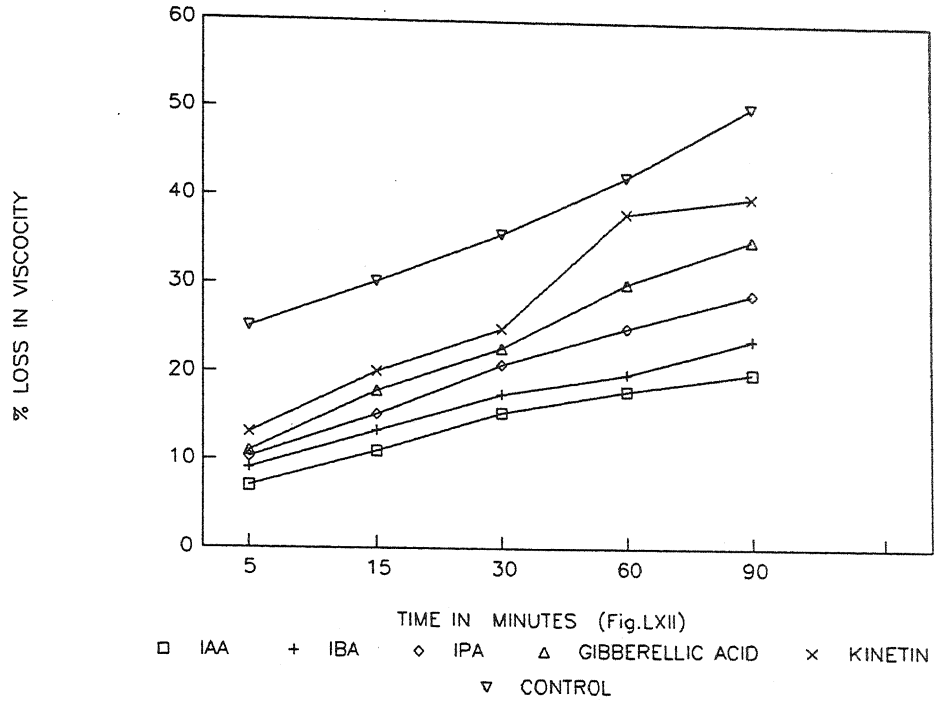
Plant growth regulators	concentration ppm	% loss in viscocity after minutes				
		5	15	30	60	90
Indole Acetic acid.	10	13.1	20.1	25.0	38.1	40.1
Indole Butyric acid.	10	7.1	11.1	15.5	18.1	20.1
Indole Propionic acid.	10	11.1	17.9	22.8	30.1	35.1
Gibberellic acid	10	9.1	13.4	17.6	19.9	23.8
Kinetin	10	10.3	15.2	20.9	25.1	29.0
CONTROL		25.1	30.2	35.6	42.2	50.3

TABLE XXXV

EFFECT OF VARIOUS FUNGICIDES ON THE PRODUCTION OF CELLULASE BY P.aphanidermatum

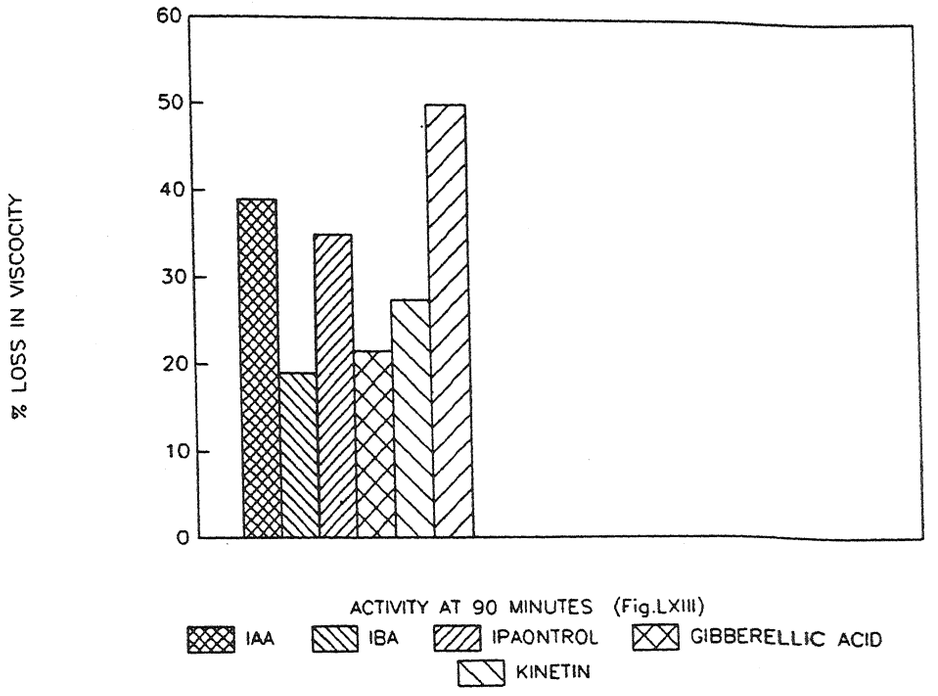
SNO	FUNGICIDES	% LOSS IN VISCOCITY AFTER MINUTES				
		5	15	30	60	90
1.	THIRAM	2.0	2.2	2.5	3.0	3.5
2.	BRASSICOL	8.1	10.3	13.8	17.1	19.7
3.	BLITANE	4.1	6.3	10.1	13.1	15.1
4.	CAPTANE	3.1	4.5	5.7	6.1	8.2
5.	CONTROL	25.1	30.1	35.6	42.2	50.3

EFFECT OF PLANT GROWTH REGULATORS ON CELLULASE PRODUCTION BY P. aphanidermatum



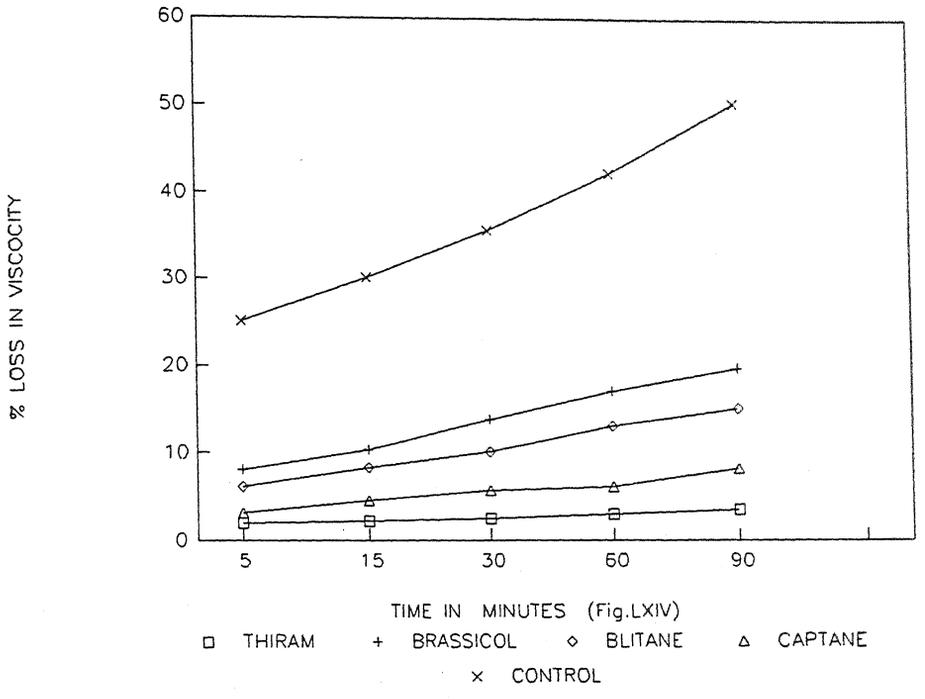
EFFECT OF PLANT GROWTH REGULATORS

CELLULASE PRODN.BY P.aphanidermatum

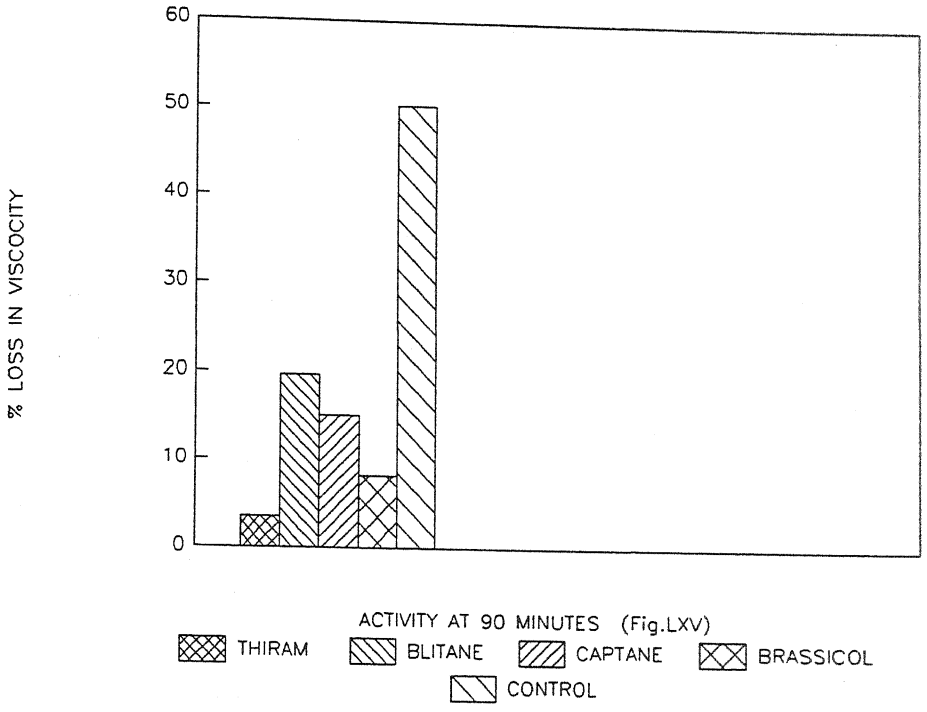


EFFECT OF DIFFERENT FUNGICIDES ON THE

PRODTN OF CELLULASE BY P.aphanidermatum



EFFECT OF FUNGICIDES ON CELLULASE PRODUCTION by P. aphanidermatum



CHAPTER XXXVII

DISCUSSION AND CONCLUSION

Cellulase production, as already been stated in the preceeding chapter is remarkably affected by different organic substances. Some increase while the other decrease. The author from the point of view of the control measure is more concerned for those substances which reduced the mycelial growth. During the course of present study the author succeeded in getting the object fulfilled by testing the effect of plant growth regulators and fungicides on enzyme production by P. aphanidermatum. The results obtained are being discussed below:

1. Plant growth regulators

Cellulase production was considerably decreased by all — the five plant growth regulators (Table XXXIV,Fig.LXII - LXIII) The maximum inhibitation was brought about by IBA followed by Gibberellic acid & Kinetin.IAA though also showed inhibitory effect but was least effective as compared to the other growth regulator. BeMiller et.al.(1969) also observed inhibition of cellulolytic enzymes of Diplodia by various concentrations of IAA. The magnitude of cellulase inhibition observed by them was very much higher than observed by the author.

2. FUNGICIDES:

Cellulase secretion and mycelial growth on broth culture was strongly suppressed by the fungicides tested i.e., Thiram, Captane, Brassicol, Blitane. Thiram and Captane were very strongly affected, Brassicol was also significantly effective in reducing cellulase production but in comparison to the other fungicides tested was least effective. (Table XXXV & Fig.LXIVLXV).

The results obtained have shown that fungicides were comparatively more effective as reducers of mycelial growth and enzyme production as compared to the growth regulators. . These results are quite similar to the results obtained by many investigators.

PART III

CONTROL MEASURES

CHAPTER XXXVIII

INTRODUCTION

During the recent years soft rot has become one of the principle diseases of the orchards and vegetable gardens. Regions of Bundel Khand under investigation in the present study proved to be seriously hit by the soft rot of ginger. It is a serious problem not because it is the principle disease of this locality infecting ginger but because these areas,particularly Barauasagar & Talbehat are the chief centres of ginger cultivation and since here it causes 80% loss to ginger production,the ginger growing farmers are shifting their cultivation for some other crop. Thus it has become a serious concern for the horticulturist of this area and on being approached by the Deputy Director, Horticulture, Jhansi, the concern was felt and the work towards this disease was undertaken by the present investigator. Much —

— about the disease has been worked out in the previous sections. This section will be delt with the control on the soft rot of ginger caused by P. aphanidermatum.. The control of disease. can be done by exploiting the environmental conditions of the pathogens through the agency of living organisms so that there is a reduction in the incidence of disease . Addition of organic materials in the form of plant residues and other organic amendments have received greater attention because of the easy ability and non toxic,from the point of view of human consumptions, handling and at the same time are relatively cheap. These amendments may be with or without nitrogen. The control measures undertaken during the present investigations by such means are being referred under 'Biological Control'.

Another method of soil borne plant disease is using chemicals. The chemicals may be organic or inorganic in nature and are by themselves responsible for reducing the growth of the pathogen.

Among the chemicals used by the author are Plant Growth Regulators and the Fungicides. These substances have a direct inhibitory effect on the growth of the pathogen, and are widely used in controlling various root diseases of crop plant. These study will be included under the sub-section "Chemical Control". Thus this section of control measure has been divided into two sections -

1. Biological control
2. Chemical Control

CHAPTER XXXIX

REVIEW OF LITERATURE

Sharma and Joshi (1975) have studied the rhizosphere microflora of ginger. Within this microflora there are parasitic,antagonistic, symbiotic and commensalic associations.In the field borne disease antagonism receives much attention and maybe due to antibiosis, exploitation or competition. Ecological significance of antibiotic production has been described by Brain (1957). Antagonistic and competitive mechanism limiting survival and activity of fungus in soil has been described by Brain (1960).

Soil antagonist play very important role in reducing root diseases specially caused by Pythiceous fungi like Pythium and Phytophthora.Katzer (1939) reported decrease of Pytophthora parasitica on tomato when Trichoderma was introduced into the soil. Grossbard (1948), (1952) found that damping off of tomato seedling caused by Pytophthora parasitica was suppressed in sterilized soil when Aspergillus clasvatus & Pencillium clavatum were introduced before inoculation of the pathogen but similar results could not be achieved by Gregory et.al.(1952); Dunleavy (1955). It was then realised that by mere introduction of antagonist in the soil,equilibrium could be disturbed temporarily and soon the former conditions are restored.Garrett (1965), Weindling & Fawcett(1936) found that damping off citrus could be controlled by T.viride only after acidifying the soil. Thus making conditions suitable for the activity of this fungus. Culture filtrates and active antagonist grown on suitable media are tried by number of workers in sterilized and unsterilized soil. Cooper and Chilton (1940), Gregory et.al,(1952), Brown (1955), Sexana (1967) but positive control to any appreciable extent could not be achieved. This is due to the fact that filtrates of active antagonist when added to the soil are often biologically degraded, physically absorbed or chemically inactivated.

Now it has been realised that mere introduction of antagonist to the soil is not sufficient to achieve disease control. Its existance in active stage is of firm importance. The soil should be added with some organic base so that the antagonistic microbial population could be indirectly boosted up. Thus, soil is added with substances which suit the growth of antagonist already present in the soil for this manuring soil amendments with organic and in organic materials have been used.

Addition of organic matter in form of plant residue have received greater attention. The subject has been thoroughly reviewed by Garrett (1956,65) and Patrick & Toussonn (1965)controlled potato scab by green manuring.Sanford (1926), Millard & Taylor (1927),

Fellow (1929) have controlled the take all disease of wheat by organic manure. Similarly biologically Wast & Thildebrand (1941) could control root rot of Strawberry; Zentmyer & Panlous (1957), Zentmyer (1963) control Phytophthora root rot of Evocodo.Davey & Papavizas (1960) obtained suppression of foot and root rot of snap beans. Zentmyer (1963) have controlled similarly root rot of Evocodo by P. cinnamoni. Reddi et.al.(1951) controlled damping off tomato seedling by adding green manure.

Fungi differ in their relative sensitivity to carbon dioxide and nitrogen .Papavizas & Davey (1959, 62) showed suppression of R. solaniby adding organic amendments. They found that low C:N ratio was effective in suppressing the pathogen. Synder et.al.(1959) found amendments of high C:N ratio such as barley straw, corn straw etc. to be effective in controlling Fusarium foot rot of snap beans. Hooker (1953) has mentioned that root rot of corn by Pythiumwas most severe in soils of low fertility. Agnihotri & Vaartaya (1967) have shown that in soil fertilised with ammonia and urea sporangia of Pythium germinate. Kauraw (1970) has concluded that root rot of wheat by P.graminicolum can be effectively controlled by using high doses of Nitrogen.Rajan & Singh (1974) has also found that nitrogen was effective in controlling tomato seedling rot of P. aphanidermatum by using high doses of nitrogen. This nitrogen not only affects the pathogen but at the same time these are helpful in increasing the yield of ginger. Muralidharan & et.al.(1973).

Soil amendments with the above inorganic substances as well as green manuring has been suggested and tried as a control for Pythium root rot. The field crops were sometimes effective mainly because they encourage soil flora antagonist to Pythium species. Apart from this, very few of these amendments also change the nature of the soil and make the physical condition unfit for P. aphanidermatum in the soil.Rajan & Singh (1973) has also observed that P. aphanidermatum was detectable only when the clay content of the soil was high. The author have also seen during the field observation that the incidence of the disease in water logged clayey soil was high thus in the present study the main stress was laid down on amendments with plant residues. These will not only alter the physical conditions but at the same time serve many purposes i.e., will increase the nutrient capacity of the soil,will effect soil C:N ratio and will boost up the antagonistic population of the soil. Thomas (1939) suggested biological control for Pythium species using Trichoderma lignorum. It increased the acidity of the medium that became unfavourable for the growth of Pythium.

Chemicals are generally used against pathogens are called "Soil Fungicides" as suggested by Domsch (1964) . The use of such chemicals for horticulture and crop control has steadily increased. Kedrik et.al.(1957),Krentzer (1961),Martin et.al.(1958),New Hall (1955)

Domsch (1964) have given detail information on different aspects of soil fungicides. Mehta (1971) in his 8th Mundker Memorail Lecture analysis the importance of fungicides for increasing the yield & control of plant diseases . He listed about 200 fungicides which are in the world market and stressed the need to devote more attention on spraying of fungicides. A number of workers have used fungicides for the control of plant diseases. Bird (1965-66) on seedling of diseased cotton,Bell (1963) on damping off of cotton, Richardson (1960) on damping off of peas . Mishra et.al.(1969) on paddy seeds , Mathur et.al.(1971) on early blight of potatoes Kushwah et.al(1971) on powdery mildue of Linn seed. Various fungicide have been studied to control plant diseases.

Abdel Kadar et.al.(1981) found Bavisten to be an effective fungicide on a number of fungus species. Padmini and Mallikarjunadhya (1985) have revealed that Bavisten at .05, .1%, 1.5% & 2% concentrations have inhibited various species of Aspergillus. Kuthubutheen and Pugh (1977) have observed the effect of Thiram and the radial growth of thermophillic fungi.

Control of P.aphanidermatum root diseases with soil fungicide has been described by Tammen et.al.(1961). Mechanism of tolerence of Pythium species to ethagol is described by Halose and Huisman (1976), Sharma & Tiwari (1975) has described the effect of chloroneb on P. aphanidermatum.

Bordeaux mixture has been recommended for P. aphanidermatum by Shahare &Asthana (1962), Bhawat (1960) recomended itfor P. myriotylum.Mercuric chloride has been recommended by Park (1935), Kothari (1966) , Thomas (1941) for various species of Pythium. These persons obtained significant control by using these above mentioned fungicides in seed soil treatment. Agarwal (1972) , Haware & Joshi (1974,-b) & Sharma & Jain (1978-a) suggested that the disease can be controlled by soaking cut rhizomes in fungicidal solution of .3% Dithane, M-45 or Benlate or Bavisten for 2 hrs followed by two drenching first at the time of sowing and second 15 days after the first drenching. Kothari (1966) used Thiram in Dethane in .2% concentration for soil drenches and Ceresan at .5 and .25% concentration for rhizome treatment. He obtained significant control by these fungicides and withMercuric chloride he obtained the highest germination. In the post harvest treatment, Haware,Joshi & Sharma (1973) found again that fungicides were useful to control the storage rot of ginger. These fungicides should be used in concentration so that they could control the occurence of ginger rot but not in such a percentage that the use may be harmful for consumption. Arora et.al.(1977) have found the persistence on some fungicides on some rhizome during storage. Sharma & Joshi (1979) have made in vitro studies of 5 fungicides against 11 seed

borne fungi and found them effective in controlling the rhyzome rot of ginger during storage. Tammen et.al.(1961) have used Captane, Ferbane, Thiram, Zeneb and certain other chemical mixed with steam treated soil and determine the effectiveness in limiting colonization of P. spinosum. The fact that Thiram, persist in soil for a short duration was varified. Captane persist in soil for 92 days and Dizon limited colonization for 92 days. Shahare & Asthana (1962) used Bordeaux mixture , Perenox and Dithane mixture to control rhizome rot of ginger caused by various species of Pythium. In the green house conditions a large number of fungicides and other chemicals have been used for soil treatment and surface dips by Scheffer & Haney (1956) . This was used against diseases caused by Pythium ,Rhizoctonia and the other fungi.

Donald Munneke (1972) has reviewed the factors affecting the efficiency of fungicides in soil. Domsch (1964) has reviewed the origin function , screening,fungi static properties of various fungicides. He has mentioned that a fungus can be easily killed in lab on artificial media or in soil in close containers or pots —

— but in the field conditions it appears to be quite different because resting structures remain viable and thus unduly concentrations are required. Rise in temperature helps in disappearance of gaseous toxicants from the soil and thus is true for most fumigants. The fungi static properties have a broad range of possible reactions. High sensitivity can be found in a group of organisms and the low sensitivity for other groups and the same is for the type of fungicide. Standard fungicides having a higher level of activity are available and are recommended for small uses. The persistance of the pesticide in the soil depends upon the physical structure of the soil for e.g., Captane is most stable in silt soil. Precipitation also change the nature of the fungicides this is also true for the influence of light. Young mycelia are more resistant to fungicides than the old ones. Starving mycelia are more sensitive than mycelia in good status. Different types of resting bodies show different sensitivity.

Angio spermic plants and fungi have many metabolic process in —

—common for this reason it is difficult to find out substance with high fungal activity and low phytotoxicity it is really useful to charecterise the relation between phytotoxicity and fungitoxicity by the so called chemotheraputic index.

Fungicides are formulated solutions, suspension, emulsion, or dust. Water soluble fungicides are not available to some extent. Some of them are rapidly decomposed or absorbed to soil. Therefore, it is recommended to use fungicides as soil mixture than as soil drench. Suspension of the fungicide are retained in the top layer of the soil, where they may

cause serious phytotoxic injuries sometimes fungicide emulsions are more effective than suspensions. Environmental factors which effects fungicides are temperature, moisture content, light, heat etc.

The fungicide has an effect on the fungus metabolism. There toxic effect has not been worked out in detail. They may effect the living cell membranes,develop an oxidation product, ,result in marked fallen respirations, starved oxygen uptake, affect carbon, reductase system of Pythium.Inhibit the pectolytic enzymes, germination of zoospores and mycelial growth may be suppressed and there may be similar large number of actions as has been mentioned by Domsch (1964).

PART III

SECTION 'A'

BIOLOGICAL CONTROL

CHAPTER XI

INTRODUCTION

Biological control as defined by Garrett (1956, 65) as "Any condition under which or practice where by, survival or activity of a pathogen is reduced through the agency of any other living organism (except man himself) with the result that there is reduction in the incidence of the disease caused by the pathogen". Efforts have been made by earlier workers to achieve biological control by direct inoculation of a suitable antagionist to the soil or on plant surface. Such change in the soil could suppress the pathogen in the soil but this would be a very short term control measure as it could alter the soil population for a short duration only. Population is the reflection of the habitate thus the author believes that change in habitates by making soil amendments with suitable plant residues with or without nitrogen could serve the purpose better.

It was realised that persistant active state of antagonist in the soil is of prime importance. Therefore, the favourable conditions to let the antagonist come in active state to change the soil environment so as to suit the growth and activity of antagonist already present in the soil. For such alteration several method as manuring, soil amendments with organic plant residues have achieved greater attention.(as such this sub-section was restricted towards soil amendments with suitable plant residues green manuring).

CHAPTER XLI

EXPERIMENTAL

The experiments in this section were performed under natural conditions. The plant residues for amending the soil were :

1. Barley straw,
2. Corn straw,
3. wheat straw,
4. Sugar cane straw

These straws have been selected with the view that these can be made easily available and at the sametime economical.

A. **Pots Experiment**:

Several pots were filled with 2 kg of air dried and sieved natural soil for each treatment . The above plant residues when added at 1% level and completely mixed with the soil in addition to the amendments of the pots in each treatment also received NH4NO3 at 200 ppm to decrease the C:N ratio. Inoculation of the pathogen at 20% was given to the pot in the form of sand oatmeal innoculum. Pathogens were added in two different ways.

1. Pathogen inoculated simultaneously to the plant residue amendments and
2. Pathogen added after incubating the amended soil for 3 weeks under natural conditions.

Control pots without amendments were run side by side. All the pots were kept under glass house conditions at $26 \pm 2°$ C and moisture content was maintained at 50% moisture capacity for one month. After these treatments the pots were sown with ginger rhizome and observed for disease incidence. The result are given in table XXXVI.

B. **Isolation of micro organisms from soil with and without plant residues:**

Plant residues with adhering soil was brought to the lab and was kept in 250 ml round bottom flask and shaken on a mechanical wrist action shaker for about 30 minutes at 200 oscillations per minute. Suitable dilutions were made from this suspension i.e., 1×10^4 for fungi and 1×10^6 for bacteria and actinomy cetes. 1 ml of this dilution was poured in petri plates and plated with Peptone Dextrose and Czapecks Dox for fungi .

CHAPTER XLII

RESULTS AND OBSERVATIONS

A. Pot Experiment:

In this experiment barley, corn, wheat and sugarcane straws were taken for soil amendments. The affect of these amendments with or without supplemented nitrogen on control of ginger rot are given in the Table XXXVI. A perusal of the table indicate that all these amendments checked the soft rot of ginger caused by P. aphinadermatum except for sugarcane where the control was not to such an extent as in case of pots where pathogen was simultaneously inoculated the disease was more.On the other hand good control of the disease was obtained in those pots which were incubated 3 weeks before inoculation of the pathogen. This is because micro organism present in the soil took some time to germinate, colonize and multiply on plant residues added to the soil after its decomposition. At the same time the pathogen when added simultaneously to the soil in active state grew faster and infected the host plants before soil microorganism could decompose and develop on the organic amendments . In amended soil supplemented with nitrogen and incubated for 3 weeks, enough time was provided to the soil microorganism to decompose and develop on plant residue in presence of freely available nitrogen and multiply on it. When the pathogen at this stage was inoculated to the soil the increased population of microorganism inhibited the growth and entry of the pathogen in the rhizome of ginger either due to completion or due to excessive production of carbondioxide or toxity of nitrogen. The best result on disease control were observed against barley straw followed by wheat and corn straw, sugarcane straw was the least effected.

B. Isolation of micro organisms from soil with or without plant residues.:

During the isolation of the micro organism from the plant residue amended soil fungal, bacterial organism and actinomytes were isolated. The percentage occurrence of these microorganism was recorded after 3 weeks of soil amendment. The data obtained are given in the table XXXVII. The highest percentage of these organism was observed in barley and corn straw manifested soil. The anticomycetes and fungi were compared to the fungal organism. Among the actinomycetes the percentage occurrence of both of them were good . However in bacteria, Pseudomonas species (isolate no. B37) occurred in a good percentage occurrence while the other two had a very low percentage occurrence. Among the fungal organism , the population of Trichoderma was the highest followed by Aspergillus terreus.

As. niger As. candidus and Penicillium chrisogenum gave a higher percentage occurrence.

C. Screening of soil organisms against P. aphanidermatum:

From the results obtained in the table XXXVII it will be clear thatby these soil amendments the population of certain organism have been boosted.The organism isolated during such preliminary isolation were subjected to their antagonistic test against P. aphanidermatum.. The results obtained are given in table XXXVIII. The table includes 22 organism which were screened against P. aphanidermatum. Out of these 15 showed various amount of inhibitory zones. Among these were 11 fungi, 2 actino-mycetes and 1 bacteria. The most prominent anatagonist were Streptomyces Species (isolate No,. A30 & 31).Aspergillus terrus. As candidus. As niger. Pencillium chrisogenum. P.funiculosum.. A few other species of Aspergillus.Pencillium and Chetomium also show moderately well antagonism. Trichoderma was found to be parasitic on P. aphanidermatum. This antagonist was fast growing thus,over runs the colony of the pathogen before any inhibition zone was observed and then the hyphae of Trichoderma coiled around those of P. aphinadermatum no haustoria was observed.

In the later stage it was found that the contents of the pathogen hyphae were empty. Trichoderma sporulates earlier at this place where it had parasitised the hyphae of the host. Soil extract medium was used for bacteria and Glucose Asparagin Agar medium was used for actinomycetes. Incubation was done at 26° C for fungi & 30° C for bacteria and actinomycetes. After 5-10 days colonies were picked up .Table XXXVII.

D. Screening of soil organisms against P.aphanidermatum

The organism isolated above were tested against P. aphanidermatum by streak method. The pathogen was streaked towards the periphery of the petridish and the test organism was streaked at right angle to it. The antagonistic activity was observed by the formation of a clear zone of inhibition developed between the pathogen and the test organism. This inhibition zone was measured with the help of a thin plastic scale. The results are given in the table XXXVIII.

TABLE XXXVI

EFFECT OF PLANT RESIDUES WITH OR WITH OUT SUPPLEMENTED NITROGEN ON THE CONTROL OF DISEASE OF GINGER RHIZOME

S.NO	AMENDMENTS	AMENDMENTS ADDED SIMULTANEOUSLY WITH PATHOGEN		AMENDMENTS ADDED 3 WEEKS BEFORE THE PATHOGEN	
		% infection	% healthy	% infection	% healthy
1.	Control(I) plant only.	4	96	2	98
2.	Control(II)plant + pathogen	80	20	70	30
3.	Control(III)plant + pathogen + N	65	35	60	40
4.	Plant + Pathogen + Barley straw.	35	65	30	70
5.	Plant + Pathogen + N + Barley straw.	40	60	25	75
6.	Plant + Pathogen + Corn straw.	35	65	41	59
7.	Plant + Pathogen + Corn straw + N.	50	50	40	60
8.	Plant + Pathogen + Wheat straw	35	65	32	68
9.	Plant + pathogen + Wheat straw + N	45	55	30	70
10.	Plant + pathogen + Sugar cane straw.	33	67	50	50
11.	Plant + pathogen + Sugar cane straw + N.	55	45	58	42

TABLE XXXVII

% OCCURANCE OF FUNGI IN PLANT RESIDUE AMENDED SOIL

S.NO	Name Of the Fungi	Isolate	PERCENTAGE OCCURANCE			
			Barley straw	Corn straw	Wheat straw	sugarcane straw
1.	Aspergillus candidus	F2	16	16	12	8
2.	As. flowus	F3	7	6	8	5
3.	As. niger	F4	10	14	12	9
4.	As. terreus	F6	16	16	16	10
5.	As.indulance	F8	12	9	8	10
6.	Cheatomium Sps	F11	4	3	3.8	2.5
7.	Penicillium chrisogen	F17	10	12	11	11
8.	P.nigricans	F18	8	8	7	5
9.	P.funiculosum	F20	10	10	8	7
10.	P.notatum	F21	8	6	7	5
11.	P.stolonifer	F22	9	8	7	8.2
12.	Fusanium okysporum	F16	8	6	5	7
13.	Cuvularia lunate	F14	3	2	1	2
14.	Absidia ramosa	F1	3	4	2	3
15.	Coanophora sps.	F12	2	2	1	1.2
16.	Cunninghamella verticelata	F13	3	2.5	1	2
17.	Trichoderma viride	F25	25	25	20	10
18.	Streptomyces sps.	A30	8	12	8	7
19.	Stretomyces sps.	A31	8	16	8	7
20.	Pseudomonas aerugenosa	B36	2	2	1	1.5
21.	P.sps	B37	12	16	12	10
22.	Erwinia carotovora	B38	2	2	1.2	1.5

TABLE XXXVIII

MICRO ORGANISM SCREENING SHOWING ANTAGONISTIC ACTIVITY

AGAINST P.aphanidermatum

S.NO	NAME OF THE FUNGI	ISOLATE.NO	ACTIVITY AGAINST P.aphanidermatum
1.	Aspergillus candidus	F2	+ + +
2.	AS.Flaris	F3	+ +
3.	As.terreus	F6	+ + + +
4.	As.indulaus	F8	+
5.	As.niger	F4	+ + +
6.	Chetomium sps.	F11	+ +
7.	Penicillium chrisogen	F17	+ + +
8.	P.nigricans	F18	+
9.	P.funiculosum	F20	+ + +
10.	P.notatum	F21	+ +
11.	P.stolonifer	F22	+ +
12.	Fusanium okysporum	F16	---
13.	Cuvularia lunate	F14	---
14.	Absidia ramosa	F1	---
15.	Coanophora sps.	F12	---
16.	Cunninghamella verticelata.	F13	N
17.	Trichoderma viride	F25	PARASITE
18.	Streptomyces sps.	A30	+ + + + +
19.	Stretomyces sps.	A31	+ + + +
20.	Pseudomonas aerugenosa	B36	---
21.	P.sps	B37	+ +
22.	Erwinia carotovora	B38	---

--- = Inhibition absent; N = Not recorded ; + = 6mm - 8mm ;

+ + = 9mm - 11mm ; + + + = 12mm - 15mm; ++++ = 16mm - 19mm;

+++++ = 20mm & above;

CHAPTER XLIII

DISCUSSION & CONCLUSION

In this section the various plants residues such as barley,. corn, wheat and sugarcane straws were used for soil amendments with or without nitrogen to find out their possible utilization as control measures for the soft rot disease of ginger caused by P. aphanidermatum. The principle behind this type of biological control was to boost up artificially the natural antagonist of the soil and make it unfavourable for the growth of the pathogen. For these control experiments residues were added at 1% level and mixed with the soil,. In addition half of the pots in each treatment also received NH_4NO_3 200 ppm to alter the C:N ratio,. Pathogen was added —

1. Simultaneously with the amendments &

2. After incubating the amendments for 3 weeks under natural conditions and in germinating rhizomes were added .

The disease incidence is given in the table XXXVI. A perusal of the table indicates that all amendments check the growth of pathogens to an appreciable extent. In the series where plants were supplemented with nitrogen and pathogen was inoculated simultaneously the disease was more sever. On the other hand good control was obtained in which the pathogen was inoculated after 3 weeks of incubation. This is due to increased microbial activity of the antagonist in the presence of available nitrogen, and sufficient time for their multiplication on the substrate. Addition of nitrogen meets the fertilizer requirement of ginger Muralidharan et.al.(1973), (1975) and suppresses the germination of sporangia of Pythium whereby serves better and healthy yield, suppresses the pathogen at the same time boost the antagonistic microflora of the soil.

On the other hand when the pathogen was added simultaneously readily infected the host plant before soil antagonist could exert their influence on its activity. The barley,wheat straw proved to be the best. These amendments control the disease incidence by making available the antagonistic flora. This was confirmed by the author by isolating organisms in the presence of these plants residues(table XXXVII)and than by screening them against the plant pathogen, P. aphanidermatum(Table XXXVIII).

The author observed that the organism which were found in higher frequency had a positive antagonistic activity against P. aphanidermatum. (Table XXXVII and XXXVIII.)This

by itself proves that these residues has harboured the antagonistic microbial flora and thereby resulted a significant control.

Apart from the role of these residues in increasing the antagonistic micro organism they also alter the physical nature of the soil. To the author's mind,this must be one of the factor responsible for the suppression of the disease as during field observation water lodged and clayey soil were found to be favourable for the disease incidence. –

–This observation is in tune with those of Rajan & Singh –

–(1973) who found detectable population of P.aphanidermatum in soil only when clay content was high,. Similarly soil amendments such as saw dust, bark and other crop residues as well as green manuring has been suggested , tried and found to be effected by many workers for the control of Pythium rot in nurseries and field crops by Sanford (1926), Millard (1927), Fellow (1929), Davey & Papavizas (1960) Zentmyer (1963). The observations were similar to those of the author.

PART III

SECTION 'B'

CHEMICAL CONTROL

CHAPTER XLIV

INTRODUCTION

Fungicide are generally chemicals used against soil pathogens. Domsch (1964) New Hall(1955) Martin et al(1958) Kretzer &Domsch (1964) have given good information on different soil fungicides. Theuse of fungicides in soil is quite different because of their hinderence in penetration and at the same time on the above ground parts they act against pathogens in more or less a mono culture like conditions. In the soil total micro flora exert a positive or negative microflora on the action of the fungicides. These fungicides sometimes cause problems. Lockwood (1964), Newhall (1955), sometimes give unexpected benefits Altman (1965), Collin's(1965).

Use of these fungicides is now increasing in various soil diseases. Their effectiveness lie on their persistence in the soil during the active stages of the pathogen growth. In soil they are generally applied in higher concentration, low concentration is helpful in cases after soil treatment with fumigations or steam but such treatments are helpful only in small scale cultures or in the glass house studies. As such treatment is not possible in Baruasagar and other large areas of Bundhelkhand which are under consideration under present studies.

The Fungicides are also applied for seed treatments before sowing Kothari (1966),Haware,Joshi & Sharma (1973), Sharma & Joshi (1979) and many other workers.Some of them have used these fungicides against the post harvest disease of ginger.

In this study well known fungicides were used for seed and soil treatment. Before attempting to use these fungicides the effect of fungicides on the radial growth of the pathogens is also studied. The experiments were planned and performed in the following steps.

1. Fungicides were studies for their effect on the radial growth of pathogen.

2. These fungicides were then used in soil to control the diseases under unsterilized conditions.3. Preseed treatment of these fungicides and were then sownto study their effects on germination and growth of the rhizomes.

CHAPTER XLV

EXPERIMENTAL

1. **Studies on the Radial growth of Pathogen**:

Fungicides were mixed with water and then sterilized. Sterilized petriplates were poured with the plain PDA to which was added 2 ml of fungicides and mixed by giving a rotatory movement. The plates were inoculated with 8 mm disc of the actively growing pathogen in the centre. Controldishes were kept without addition of any fungicides.Triplicates were taken for each fungicides. These dish-es were incubated at 26° C. The diameter of the colony was measured after every 24 hrs with a plastic scale for 4 days.

2. **Experiment in soil under unsterilized condition**:

Earthen pots of 2 kg capacity were used. Each pot received1 kg air dry soil of infected area and 4 rhizomes. Requisite quantity of fungicidal solution in water was added to make the moisture content 50%. These pots were also placed on troughs with one inch level of water. Loss of water if any was added from time to time. Pots with no fungicidal solution were run as control.

3. **Experiment with Pre-treatment of seeds**:

Earthen pots of 2 kg capacity containing soil of the infested area were used to grow 4 seeds (20 such pots were taken for such treatment) in each pot seeds were washed and previously soaked in fungicidal solution for 5 minutes. Seeds treated with distilled water were grown as control .(Control pot received garden soil). Here pots were irrigated as and when necessary. Observations were made from the time of germination to 3 weeks old plants. Effects were noted on percentage germination, height of the plant and the relative size of the leaf and their root system.

CHAPTER XLVI

RESULTS AND OBSERVATIONS

1. **Studies on the radial growth of pathogen** :

Before the studies on the radial growth of P.aphanidermatum fungicides were bio assayed for their effect on the growth of the pathogen. Forthis 10 fungicides were used, these were Cosan, Cuman, Blitane, Brassicol, Blitox,Sultaf, Thiovit, Thiram, Captane, Blimix. All these fungicides were used in .5% concentration in water . During this preliminary screening the pathogen was grown in petridish and,sterilized filter paper disc soaked in fungicides where used to see that inhibitory effect.

Out of these fungicides, 4 fungicides were selected for further studies. These were Blitane, Captane, Thiram, & Brassicol. These 4 fungicides were used for their effect on the radial growth of the pathogen. Results obtained are given in the table XXXIX. The results confirm the observation found in the preliminary screening. In this experiment both Thiram & Captane produced significant activity.Blitane was next to Thiram and Captane for its inhibitory effect and Brassicol did not produce any effect, the pathogen grew normal as growing in control.

2. **Control experiment in soil**:

In this experiment Thiram & Captane were used to control the soft rot of ginger caused by P.aphanidermatum . The concentration selected for these fungicides were 1% for Thiram and .5 % for Captane. These two concentration were thought to be suitable under natural conditions. Among these two fungicides used Thiram was found to be more effective and gave 73-75% control, while in Captane 65-68% control was observed which is shown in table XL. They could have produced better results if used in higher concentrations. But higher concentration was thought to be toxic to the crop root and was not used. The soil when plated in Thiram,infested soil,showed the presence of Trichoderma viride.

This indicates that Thiram also stimulated the active antagonist . From the above result, it could be said that Thiram and Captane could give a fair suppression on the soft rot of ginger and could be an effective control if used under field conditions.

3. **Pre-seed treatment**:

In this experiment the same two fungicides used in the above experiment were studied. The two fungicides used above were Thiram in the concentration of .5% and .25%, and Captane in a concentration of .25% and .125%. The percentage germination of seed was

observed and tabulated in the table XLI. The results show that for seed treatment Thiram gave good results in .5% concentration and Captane in .25% concentration. Lower concentration of both the fungicides were also effective, but less effective as compared to the higher concentration. Thiram was more effective than Captane as it gave 81.2% germination as compared to Captane which gave 77.5% germination. Control experiments using garden soil gave 88.8% germination.

TABLE XXXIX

EFFECT OF FUNGICIDES ON THE RADIAL GROWTH OF THE PATHOGEN

Sno	Name of the Fungicide	No of days after inoculation					Average growth/day
		initial	1st day	2nd day	3rd day	4th day	
1.	Blitane	0.8cm	2.5cms	5.0cms	7.6cms	full	2·3 cm
2.	Brassicol	0.8cm	4.0cms	6.1cms	full	full	1·04 cm
3.	Captane	0.8cm	0.8cm	0.8cm	0.8cm	0.8cm	nil
4.	Thiram	0.8cm	0.8cm	0.8cm	0.8cm	0.8cm	nil
5.	Control	0.8cm	4.1cms	8.0cms	full	full	1·04 cm

TABLE XL

EFFECT OF FUNGICIDES ON THE DISEASE INCIDENCE BY P.aphanidermatum

SNO	Treatment	Dilution	% germination	Remarks
1.	Thiram (control 1) With infested area soil.	1%	75%	Some plants show toxic effect.
2.	P. aphanidermatum (control 2)	-----	30%	plant suffered from soft rot of ginger.
3.	Pythium + thiram	1%	73%	soft rot not observed
4.	Captane (control 1) with infested area soil.	.5%	68%	Plant showed toxic effect,root tips appearing black
5.	Pythium + Captane	.5%	65%	Plant without sign of Disease.
6.	Neither Fungicide Nor Pathogen (control 3). With garden soil	-----	90%	No disease, plants healthily and normal.

TABLE XL I

Effect of Fungicide as percent germination of ginger plant

Treatment	% concentration	No of Rhizomes treated	No plants germinated	Germination percentage
Thiram in Infested field soil.	.5%	80	65	81.2%
Thiram in infested field soil.	.25%	80	60	75%
Captane in infested field soil.	.25%	80	62	77.5%
Captane in infested field soil.	.125%	80	58	72.5%
CONTROL in GARDEN SOIL	-----	80	71	88.8%

CHAPTER XLVII

DISCUSSION AND CONCLUSION

Fungicides with higher efficiency were tested to give chemical control against soft rot of ginger. Before using these fungicides, screening was observed in bio assay experiments using filter paper disc and then radial growth of pathogen was observed on the most effective fungicides.

1. Selection of fungicides

10 fungicides were bio assayed,out of these 3 gave significant activity. These were Blitane, Captane and Thiram. These to gather with Brassicol were further used for the radial growth of pathogen. Observation on the radial growth after every 24hrs to 4 days are given in the table XXXIX. A perusal of the table indicate that Captane and Thiram were more effective in which the pathogen could not at all develop.

2. Control by selective fungicides in soil

The above experiment indicated that Blitane, Captane and Thiram gave good results. Out of these Thiram and Captane were used for further experiments as they gave best results. The concentration used for soil experiments was 1% for Thiram and .5% for Captane.Thiram gave 75% germination while Captane gave 68% germination when used in infested soil. When used in lab infested P.aphanidermatum soil Thiram gave 73% germination and Captane gave 65% germination. These results are quite encouraging when compared to the control in which garden soil was used and 90% germination was obtained.(Table XL) In this experiment the concentration used for Thiram and Captane showed slight toxic effect and therefore in the Pre seed treatment experiments concentration is further reduced.

3. Pre seed Treatment

In this experiment again only two fungicides Thiram and Captane were used. Twenty pots having 4 seeds each was studied for each treatment. Thiram gave better results than Captane and .5% concentration of Thiram gave better results than its .25% while Captane in .25% concentration gave better control as compared to its .125% concentration.(Table XLI) Thiram and Captane both can be recommended of the seed treatment and soil treatment in .5% and .25% concentration . In field soil drenches could be dug in between the lines of ginger cultivation and these fungicides could be applied. Thiram was used for soil drenches and rhizome treatment by Kothari (1966) He also observed similar degree of control

as was observed by the author. In control experiments the garden soil was used. The disease does not appear and 90% germination was obtained,which was due to the fact that ginger was grown in garden soil for the first time and there was no in-oculum potential pre existing in such soil. This observation of the author also points towards the soil dwelling nature of .aphanidermatum.

Thiram persist in soil for a short period of time and Captane persist for 92 days . Tammen et,al.(1961). This shows that in soil Captane will give better results for a longer period of time.However, in the present studies where the % germination and disease incidence was noted for 3 weeks time Thiram gave better results as compared to Captane. The results obtained by the author by using Captane were similar to those found by Scheffer and Haney (1956). Hendrix and Campbell have even suggested that Thiram and Captane mixture have given excellent control of P.aphanidermatum.

The over all control measure suggested that the following step could help the cultivation of disease free ginger crop.

Fresh healthy and disease free rhizomes should be sown for ginger cultivation.

The seed rhizome should be given minimum cut ends as they help in colonization of the pathogen. Fungicides like Thiram .5% and Captane .25% should be used for seed treatment before sowing. Deep summer ploughing should be practiced as it reduces the inoculum of the soil and water logging.

Continuous crop in the same field should be avoided as it develops inoculum in the soil. When ever possible use some other rotational field crop.

Plant residues before sowing in such fields should be added with biological control and will avoid the use of fungicides.

Sick soil previously croped with ginger must receive some, amendments like barley,wheat, corn straw, to boost up the antagonistic micro organism and to alter the physical nature of the soil.

In the sick fields the fungicides,Thiram and Captane should be used in .5% and .25% solution in water & poured in drenches first at the time of sowing and second 15 days after to keep the fungicidal activity and 3rd should be done if the disease is observed further.

The sowing should be in the third or fourth week of June i.e. before rain. Late sowing delays germination and make them vulnerable to infection due to rains.

A strong antagonist Trichoderma, may be used to control the disease by either inoculating it or increasing the antagonist by inducing organic amendments.

PART IV
MISCELLANEOUS
GENERAL SUMMARY

CHAPTER XLVIII

PART I

PATHOGENESIS

Soft rot of ginger has been reported much earlier by Butler (1907) then by many other workers from various parts of India. Subramaniam (1919) Kothari (1966) Haware & Joshi (1972) Indrasenan & Paily (1973). But it appeared in an very epedimic form in the Bundelkhand region nearby Jhansi Division. The loss has been found by the author to be nearing about 80% of the annual yield of ginger. During the isolation studies the author isolated Pythium aphanidermatum (Edson) Fitz from infected rhizomes. Symptoms appeared first on leaves followed by paleness at the basal portion the rhizome showing destruction of parenchymatous tissues and finally exudation of watery mass , lateral roots also rotten and soften.The disease was noticed to be more abundant on water logged situation where clay fractions was more. These observations were similar to those observed by Rajan & Singh (1973).The author isolated the pathogem on PDA, PDA with piramycin and vancomycin. The same organism causing soft rot was also isolated by Shahare & Asthana (1962) Park (1934, 41) Indrasenan & Paily (1973) . Pathogenisity experiments were performed by the author in culture tubes and in soil under sterilized as well as unsterilized conditions. Results of these experiments are given in Table I. The author observed that freshly developed rhizomes were more suspectible to infection as compared to the old ones.80% of the fresh rhizomes showed disease incidence after 5 days inoculation while it was only 15% disease incident on old rhizomes. However the percentage of disease incidence increase after 8 days of incubation. Glass jar experiments with sterilized and unsterilized conditions (Table II) confirm the culture tube experiments. It was also observed that pricked rhizomes gave better results as compared to unpricked ones. These observations are also in confirmity to those of the above workers.

To study the effect of culture media on the growth of the pathogen ,Potato Dextrose,Peptone Dextrose, Czapecks Dox, Glucose Asparaginand Richards media were used. The experiment were used on broth as well as on solid media and results are given in Table III. The dry mycelial weight on PD , Czapecks Dox, Peptone Dextrose and Glucose Asparagin were 295, 130, 90 and 5 mg respectively . In solid media radial growth was recorded after every 24 hrs to 72 hrs. and was incubated after 0, 24, & 48 hrs. Results are given in Table IV and Fig. I,II & III. The growth pattern shows that with the increase in the incubation period before inoculation the growth slowed down.It was fastest in PDA

followed by Czapecks Dox and Peptone Dextrose.Pathogens behaviour towards the incubation temperature of 15° C, 25° C, 35° C, 45° C was studied. The results obtained are given in table VI and Fig.IV. It was found that optimum growth was observed at 25° C and minimum around 45° C. The optimum temperature was low on liquid medium as compared to the agar medium.

C:N ratio was altered and the effect was observed on the growth of P.aphanidermatum. The experiment was conducted on Czapecks dox medium in which during one set of experiment NaNO3 was kept constant and Sucrose was changed i.e., 15gm, 30 gm & 45 gms/litre, in other set sucrose was kept constant and NaNO3 was changed i.e., 1g, 2gms & 3gms respectively. The optimum growth was obtained in the medium with 2gm/litre NaNO3 and 30gm/litre sucrose (table V and Fig. V). When the concentration of the nitrate was increased or sucrose was decreased the growth decreased. Such observations also corelates with the results observed by Agnihotri & Vaartaja (1967), Sen & Shrivastava (1968).

The age of culture has been found to be an important factor in the disease incidence. During its study as observed in Table VII cultures were raised and inoculated after 3,5 & 8 days respectively. 3 days old culture was found to be most effective and caused 75% disease. The disease incidence lowered as the culture grew old ,with 8 day old culture only 30% loss was observed. Indrasenan & Paily's (1973) experiment gave max. infection in 2 days old culture. The age of the rhizome was similarly found to be effective. The observation can be made from table I, II & VI. This showed freshly developed rhizomes and buds are more suceptible to infection as compared to old and mature rhizomes..pa

PART - II

ENZYMATIC STUDY

SECTION - A

In this part both pectic and cellulolytic enzymes developed by P. aphanidermatum has been studied in vivo,on healthy and diseased tissues,and in vitro under different cultural condition. and in presence of growth regulators and fungicides. In Section "A" pectic enzymes were studied which was divided in 3 subsection. Sub-section "a" comprises of in vivo study of pectic enzymes in healthy as well as P. aphanidermatum infected rhizomes. In healthy rhizomes four pectic enzyme were present namely PG, PMG PGTE & PMTE. However slight activity of PME was also observed. The observations are given in the Table VIII - XIII and Fig.VI - XIII. PG was found to be more active than compared to the others . PG activity in healthy fruits like Papaya is also found by Rai (1971). During the study for pectic enzymes

in diseased rhizomes all the 6 enzymes studied were found to be present. The diseased tissues showed greater enzyme activity as compared to the healthy tissue. Such higher degree of pectolytic enzyme in diseased tissue has also been observed by Ali (1970) Rai (1971),

Kretzer (1951) Bateman & Millar (1966) Indrasenan & Paily (1982). The observations of Winstead & Mc Comb (1961) also point to the same conclusions. Protopectinase , PG and PME showed a higher activity after 5 days of incubation as compared to 10 days of incubation . However, Enzyme activity of PMG, PMTE and PGTE was higher on 10 days of incubation as compared to 5 days of incubation. This shows that glycosidases are more active than trans eliminases during pathogenesis.

The In vitro studies of P. aphanidermatum were carried out for Protopectinase , PME, PG, PMG, PGTE and PMTE on Potato Dextrose, Czapecks Dox, Peptone and Glucose Asparagin media. Datas are recorded in the table XIV - XVIII.and Figure XIV - XXIX ,for 5 days and 10 days of incubation.The result show that activity of these enzymes were variable. Protopectinase was more in 5 days of incubation as compared to 10 days. Maximum activity was recorded on Potato Dextrose followed by Czapecks Dox, . Peptone Dextrose and Glucose Asparagin media. No PME activity was observed in culture filtrates either after 5 days or 10 days of incubation. These observations of the author are similar to those of Winstead and Mc Comb (1961) and Indrasenan & Paily's (1982) . PG activity was found to be maximum on Glucose Asparagin and Peptone Dextrose media followed by Czapecks Dox. Least activity was in Potato Dextrose media. The activity was more after 5 days of incubation as compared to 10 days except in Glucose Asparagin where more activity was found after 10 days of incubation. These observations are similar to Indrasenan and Paily(1982). However they noticed maximum activity on Conn's medium. PMG activity was found to be favoured most in Czapecks Dox after 5days of incubation and in Glucose Asparagin after 10 days, with regards to trans-eliminases. Glucose Asparagin produce maximum PGTE followed by Peptone Dextrose. Here the activity was higher in 10 days of incubation as compared to 5 days. PMTE production was also found to be maximum in Glucose Asparagin followed by Czapecks Dox and Peptone Dextrose. Here again, 10 days of incubation gave better enzyme activity as compared to 5 days incubation period. These results show that the enzyme vary in activity with reference to various media used for cultural studies. On the basis of these results no definite conclusions –

– could be derived as far as media is concerned because a number of factors and mechanisms are involved simultaneously in the secretion of enzymes. However, it can be mentioned that 10 days incubation could be concerned to be optimum for trans eliminase activity and

5 days for glycosidases. Mycelial growth during the observations were found to be in varying degrees in various filtrates and there can be no correlation between the mycelial growth and the enzyme production, Indrasenan & Paily (1982) Ali (1970) and Rai (1971) also had similar observations.

During the study on the cultural conditions the effect of pH on pectic enzymes production was analysed and it was found that glycosidases favour the acidic pH while the alkaline pH supported the trans eliminases. Thus pH can be said to be playing a significant role in the production of pectic enzymes by P. aphanidermatum as shown in Tables XIX - XXII. and fig XXX - XXXVII Glycosidases gave an optimum value at pH 5 and trans eliminases at pH 8.

The in vitro production of pectic enzyme was studied in the presence of growth regulators and fungicides. 5 growth regulators Indole Acetic Acid, Indole Butyric Acid, Indole Propionic Acid, Gibberellic acid and Kinetin were used in a concentration of 10 ppm while among fungicides Thiram, Brassicol, Blitane, and Captane were used in .5% concentration. Both were added to Glucose Asparagin medium. The results were observed after 5 days of incubation period and are given in Table XXIII to XXVI. fig. XXXVII - XLV .

The results obtained show that all the five growth regulators caused varying degree of inhibitory effect on the various pectic enzymes. The PMG and PMTE were more adversely effected than PG and PGTE. The synthesis of PG was strongly suppressed by IPA & Kinetin. PMG was most adversely effected by Kinetin and IPA. IAA was least affected in this case. In case of PGTE Gibberellic acid and Kinetin caused a greater inhibition of enzyme synthesis. PMTE was however more strongly suppressed by IBA followed by Gibberellin . IAA was least effective. Here again no correlation could be drawn as regard to the fungal growth and pectic enzyme secretion in the presence of various growth regulators . Conclusively Kinetin and Gibberellic Acid were more affected than the others in reducing the pectic enzyme secretion. Bateman (1966) has attributed plant disease control of some of these hormones . The mechanism of their involvement in reducing the enzyme synthesis is not clearly understood but it could be suggested that these growth regulators could act as an effective controlling agent during pathogenesis.

The results obtained from the Table XXVII - XXX and Fig. XLVI - LIII,the affect of fungicides on pectic enzyme production could be studied. The polygalactonase enzyme is strongly suppressed by Thiram and Captane. Regarding trans eliminase enzyme PMTE was also significantly affected by both Thiram and Captane. However for PGTE , Thiram was more effective as compared to Captane. Conclusively ,it can be said that both Thiram and

Captane were most effective in reducing the enzyme production. Similar inhibitions of pectic enzymes by various fungicides have also been reported by Grover & Moore (1962) & Grover (1964).

SECTION B

In this section cellulolytic enzymes were studied in vivo and in vitro, under various cultural conditions of media, incubation, and pH in presence of growth regulators and fungicides.

During the in vivo studies of healthy and P.aphinadermatum infected rhizomes, the cellulase activity in diseased rhizome was found to be slightly higher than the fresh rhizomes after 5 days of infection. As the infection period increased for 10 days cellulase activity increased (Table XXXI Fig. LIV & LV). This indicates that cellulase enzymes are involved in pathogenesis. Bateman (1964) has also recorded the presence of cellulase activity during pathogenesis. Similar observations was also recorded by Rai (1971).

The effect of different culture media on the production of cellulase by P. aphanidermatum has been given in the table XXXII fig.LVI - LIX . The cellulase production was variously favoured by different culture media.

The amount of activity varied with the incubation period. Glucose Asparagin was most favourable followed by Peptone Dextrose, Czapecks Dox and Potato Dextrose was least favoured. Ali (1970) and Rai (1971) found very little cellulase activity on PDA . When the incubation period was increased from 5 to 10 days the enzyme production increased in Glucose Asparagin and Potato Dextrose while in Czapecks Dox and Peptone Dextrose the enzyme activity was almost the same and was not much affected by increasing the incubation period. Mycelial growth during these observations was found to be maximum on PDA and minimum on Glucose Asparagin . Thus no correlation between enzyme production and mycelial growth could be drawn.

For the study of effect of pH on cellulase production Glucose Asparagin media was used and as it gave more activity after 10 days of incubation, the observation for variable pH was also done on 10 days old culture. From the result shown in the Table XXXIII , Fig. LX & LXI it was observed that optimum cellulase activity was there at pH4 and the favourable range was between 3-5 . No cellulolytic activity was found below pH3, and above pH5 cellulase activity progressively decrease from pH5 -pH8. Similar results were obtained by Ali (1970) Rai (1971) and Rees (1965).

During the invitro studies on cellulolytic enzyme in the presence of growth regulators and fun-

gicides it was observed that both were reducing cellulase activity .Table XXXIV & XXXV.Fig. LXII - LXV. The datas shown in table XXXIV indicate that all the 5 growth regulators considerably reduce the cellulase production. Maximum reduction was found with IBA followed by Gibberellic acid and Kinetin . Indole acetic acid showed the least inhibitory effect. During the course of study of cellulase it was also found by the that a fair amount of growth of P. aphanidermatum occurred . It appears that the toxic effect of these regulators were less pronounced on the mycelial growth as compared to the cellulase production.

Fungicides reduce considerably cellulase enzyme production as is evident from the data recorded from the table XXXV. From these datas it will be clear that all the fungicides very effectively reduce the cellulase production. Maximum inhibitory effect was found with Thiram and Captane followed by Blitane and Brassicol. Very slight mycelial growth was obtained in Thiram and Captane. Brassicol was not all toxic for the mycelial growth however it significantly reduce the cellulase production. From these results it was observed that fungicides were more effected as compared to the growth regulators in reducing the mycelial growth as well as enzyme production of P. aphanidermatum. Therefore fungicides were considered for the detailed control measures experiments in the succeeding part of the present study .

CONTROL MEASURES

SECTION - A

In this section various plant residues such as Barley, Corn , Wheat and Sugarcane straw were used with and without nitrogen to find out a possible role in controlling soft rot of ginger caused by . aphanidermatum. The principle behind was to boost up the natural antagonists present in the soil . Residue were added at 1% level and half of the pot in each treatment received NH4NO3 at 200 ppm concentration. Pathogen was added simultaneously with the amendments after incubating the amendments for 3 weeks under natural conditions. The disease incidence are recorded in the table XXXVI. The results indicate check to the disease to an appreciable extent. The disease was more severe when nitrogen and pathogen were added simultaneously. On the other hand a control was obtained when pathogen was inoculated after 3 weeks of incubation. This is due to the fact that microbial activity of the antagonist increase in the presence of nitrogen and had sufficient time for multiplication on the substrate. On the other hand when the pathogen were added simultaneously the pathogen developed readily and infected the host plant before soil antagonists could exert their influence. Barley and Wheat proved to be the best while Sugarcane was the least effective in controlling the disease incidence. This observation was confirmed by the observation in the next experiment conducted to isolate the organisms present on the plant residues in the Table

XXXVII . In a next experiment the author screened the microorganisms obtained in table XXXVII against the plant pathogen. The results of the antagonistic screening experiments in Table XXXVIII show that the organisms which were found to be in higher frequency in the isolation experiments (Table XXXVII) gave a positive antagonistic activity. This itself proves that these residues harbour the antagonistic microflora and there by contributed significantly for the control Trichoderma was found to be parasitic on P. aphanidermatum over grow the colony of the pathogen coiled around them and sporulated earlier at these places. The hyphae of P. aphanidermatum were found to be -

- empty at these places however, no haustorial structures were recorded.

Apart from the role of these residues in increasing the antagonistic microflora they alter the physical nature of the soil and at the same time add the nutrients in the soil. These factors must also be considered as alteration of physical condition from water lodged to aerated soil makes it unfavourable for the growth of P. aphanidermatum . This observation of the author is also similar to those observed by Rajan & Singh (1973). Addition of organic fertilizershave also been suggested to increase ginger yield by Muralidharan et al (1973) . The suppression of sporangial germination of Pythium by nitrogen has also been reported by Muralidharan et al (1973).

Such amendments has also been used to control the root rot disease of field crops by Sanford (1926),Millard (1927),Fellowe (1929),Davey & Papaviazas(1960)&Zentemyer (1963).

SECTION - B

In this section control with fungicides have been tried for the disease incidence on ginger rhizome by P. aphanidermatum. 10 fungicides were bio assayed for inhibitory effect on the growth of P. aphanidermatum out of these Blitane, Captane, &Thiram, significantly affected the growth of the pathogen. These fungicides together with the Brassicol was further used for their effect on the radial growth of the pathogen. Results of these are given in Table XXXIX. The table indicated that Captane and Thiram both were most active in which the pathogen could not develop at all. Control experiments were carried out in earthen pots of 2 kg capacity in which the Thiram and Captane were added to the soil . Captane was added in the .5% concentration and Thiram in 1% concentration were added to the infested area soil. The results obtained in this experiment are given in table XL. These results show that Thiram gave 75% germination while Captane gave 65% germination when used in infested field soil. When these fungicides were used with lab infested P. aphanidermatum. Thiram gave 75% germination and Captane gave 68% germination. These results were quite encour-

aging when compared to the control in which garden soil was used . In this control experiment 90% germination were obtained this was due to the fact that ginger was grown in the garden soil for the first time and there was no innoculum potential pre-existing in such soil. This observation also points towards the soil dwelling nature of P. aphinadermatum.

The next experiment was conducted to see the effective role of these fungicides when pre treated before sowing. 20 pots having 4 seeds each were studied for such treatments. Thiram gave better results than Captane. The observations were conducted in .5% and .25% concentration for Thiram and .25 and .125% concentration for Captane. The results obtained are given in table XLI. From these results Thiram and Captane both can be recommended for pre seed treatment and soil treatment at .5% and .25% concentration resp. In the fields for the use of these fungicides soil drenches could be dug between the lines of cultivation and can be applied at the time of sowing and repeated after 15 days, particularly in Pythium infested area.

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